A STUDY ON CALCIUM HOMEOSTASIS ABNORMALITIES IN PATIENTS TAKING CHRONIC ANTICONVULSANTS THERAPY IN GGH, CHENNAI

Dissertation submitted in partial fulfillment of requirements for

M.D. DEGREE IN GENERAL MEDICINE

BRANCH I

Of

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI, INDIA.

MADRAS MEDICAL COLLEGE,
CHENNAI - 600003
APRIL 2011

CERTIFICATE

This is to certify that the dissertation entitled ***"A STUDY ON CALCIUM HOMEOSTASIS ABNORMALITIES IN PATIENTS TAKING CHRONIC ANTICONVULSANTS THERAPY IN GGH, CHENNAI"*** is a bonafide work done by **Dr.N.Govindarajan**, at Madras Medical College, Chennai in partial fulfillment of the university rules and regulations for award of M.D., Degree in General Medicine (Branch-I) under my guidance and supervision during the academic course period 2008-2011. I forward this to the Tamilnadu Dr.M.G.R Medical university, Chennai, Tamilnadu, India

Prof. S.TITO, M.D.,
Professor and unit chief
Institute of Internal Medicine,
Madras Medical College &
Govt. General Hospital,
Chennai – 3.

Prof. C.RAJENDRAN, M.D.,
Director and professor
Institute of Internal Medicine,
Madras Medical College &
Govt. General Hospital,
Chennai – 3.

Prof.J.MOHANASUNDARAM, M.D,DNB,Ph.D.,
THE DEAN
Madras Medical College &
Govt. General Hospital,
Chennai – 600 003

DECLARATION

I solemnly declare that the dissertation entitled **"*A STUDY ON CALCIUM HOMEOSTASIS ABNORMALITIES IN PATIENTS TAKING CHRONIC ANTICONVULSANT THERAPY IN GGH, CHENNAI*"** is done by me at Madras Medical College, Chennai-3 during the academic course period April 2008 – April 2011 under the guidance and supervision of **Prof.S.TITO, M.D.,** to be submitted to The Tamilnadu Dr M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D DEGREE IN GENERAL MEDICINE BRANCH-I.

Date:
Place:

Dr. N.GOVINDARAJAN
M.D. General Medicine
Postgraduate Student,
Institute of Internal Medicine,
Madras Medical College,
Chennai.

ACKNOWLEDGEMENT

I thank **Prof.J.MOHANASUNDARAM, M.D.,DNB.,Ph.D.,** Dean, Madras Medical College, for having permitted me to conduct the study and use the hospital resources in the study.

I express my heartfelt gratitude to **Prof.C.RAJENDIRAN, M.D.,** Director, Institute of Internal Medicine for his inspiration, advice, and guidance in making this work complete.

I express my deep gratitude to my chief **Prof. S. TITO, M.D.,** Additional Professor, Institute of Internal Medicine for his comments, corrections and guidance to complete the study.

I am extremely thankful to Assistant Professors of Medicine **Dr. G.SUBBARAGHAVALU, M.D., and Dr.C.SRIDHAR, M.D.,** for their co-operation and guidance.

Last but not the least, I would wish to thank all the patients without whose kind cooperation, this study would not have been possible.

CONTENTS

ABBREVATIONS

ALP	-	Alkaline Phosphatase
PTH	-	Parathyroid Hormone
Vit D	-	Vitamin D
$1,25(OH)_2D_3$	-	1,25,Hydroxyl Vitamin D
AEDs	-	Anticonvulsant Drugs
EEG	-	Electroencephalogram
DBP	-	Vitamin D Binding Protein
CKD	-	Chronic Kidney Disease
INH	-	Isoniazid
IU	-	International Unit
ADH	-	Antidiuretic Hormone
CT	-	Calcitonin
GGT	-	Gamma Glutamyl Transpeptitase
RANK	-	Receptor Activator Of NF-Kappa-B
RANKL	-	Receptor Activator Of NF-Kappa-B Ligand
M	-	Meningitis
PSS	-	Post stroke seizure
N	-	Neurocysticercosis

MR	-	Mental Retardation
TBM	-	Tuberculous meningitis
CPS	-	Complex partial Seizure
PTS	-	Post traumatic seizure
A	-	Absense Seizure
I	-	Idiopathic seizure
P	-	Phenytoin
S	-	Sodium Valproate
PB	-	Phenobarbitone
C	-	Carbamazepine
Y	-	Good seizure control
N	-	Poorseisure control

INTRODUCTION

INTRODUCTION

Epilepsy is one of the well known disease entities causing significant morbidity. The staggering incidence and prevalence of 5.35/1000[1] and 49.5/100,000[1] respectively in India, enhances its importance as a health issue which needs to be studied extensively. More so, since the treatment involves long term usage of drugs known to produce significant adverse effects. Hence, being in the era of evidence based and preventive medicine, it is the duty of the medical fraternity to evaluate the possibilities of preventing such adverse effects and reducing morbidity as much as feasible.

It is well recognised that chronic anticonvulsant drug therapy produces significant disorders of bone and mineral metabolism. In various anticonvulsant drug treated populations, the reported incidence of hypocalcemia, reduced serum 25-(OH) vitamin D concentration, elevated serum immunoreactive parathyroid hormone concentration, reduced bone mass, and histologic evidence of osteomalacia varies from 10-60%.[2]

Hence it becomes clear that epilepsy and the complications induced by its treatment are present in such high proportions in our community that it is difficult to be ignored. This study is an attempt at

evaluating the presence of the above mentioned complications along with the possible pathophysiology supported by biochemical evidences.

Current evidence indicates that anticonvulsant drugs induced alterations in the hepatic metabolism of vitamin D or its biologically active metabolites, plays an important role in the pathogenesis of this disease. The reduced 25- hydroxy vitamin D concentrations observed in this disorder are attributed variously to increased hepatic microsomal catabolism of vitamin D and 25 hydroxy vitamin D or to a direct inhibition of hepatic conversion of vitamin D to 25 hydroxy vitamin D.

Investigations done in a large numbers of epileptic patients demonstrated that frank osteomalacia or rickets is relatively uncommon. By using photon absorption measurements, a moderate degree of bone demineralisation can be detected in most epileptic patients on chronic anticonvulsant drug treatment.

The laboratory abnormalities most commonly seen are hypocalcemia, hypocalciuria, reduced 25 hydroxy vitamin D as well as elevated alkaline phosphatase and immunoreactive parathyroid hormone in serum[(3)]. Based on clinical observation it appeared likely that the severity of the drug induced calcium and bone metabolism disorder depended on several factors like vitamin D intake, sun light

exposure, physical exercise, anticonvulsant dose, duration of therapy, and perhaps individual susceptibility.

Hypocalcemia induced by anti convulsants, apart from causing bone abnormalities can also cause seizures per se. Hypocalcemic seizures are uncommon and under diagnosed complication of long-term therapy with AEDs. Loss of seizure control in a patient stabilized on AEDs is an indication to check the patient's calcium status. Proper treatment of this complication is vitamin D and calcium supplementation. Prophylactic supplementation with vitamin D is necessary in institutionalized patients treated with AEDs.

If the patient has pre-existing subclinical epilepsy, hypocalcemia may lower the excitation threshold for seizures. Electroencephalographic changes may be acute and nonspecific or present with distinct specific alterations. EEG changes may be present with or without symptoms of hypocalcemia. Some patients on anticonvulsive therapy have not needed medication after appropriate treatment of their hypocalcemia. The relationship between calcification of basal ganglia, cerebral cortex, or cerebellum with pre-existing epileptic or convulsive disorders is not well understood. In the elderly

population, disorientation or confusion may be manifestations of hypocalcemia.

Hence, evaluation of these anti convulsant induced complications assumes importance in view of their treatable nature, which forms the rationale of my taking up this study.

AIM AND OBJECTIVES

AIMS AND OBJECTIVES

- To study the prevalence of calcium homeostasis abnormalities in patients on chronic anticonvulsant therapy.
- To assess the serum vitamin D level in patients on chronic anticonvulsant therapy.
- To study the relationship between the seizure control and hypocalcemia.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

VITAMIN D

Vitamin D is a fat soluble vitamin. It resembles sterols in structure and functions like a hormone. Vitamin D was isolated by August (1931) who named it calciferol. Vitamin D plays an essential role in calcium homeostasis and bone metabolism, induction of cell differentiation, inhibition of cell growth, immunomodulation, and control of other hormonal systems.

Metabolism of Vitamin D

Vitamin D is not a true vitamin, since nutritional supplementation is not required in humans who have adequate sun exposure. When the skin is exposed to ultraviolet radiation, the cutaneous precursor of vitamin D, 7-dehydrocholesterol, undergoes photochemical cleavage of the carbon bond between carbons 9 and 10 of the steroid ring. The resultant product, Previtamin D, is thermally labile and over a period of 48 hours undergoes a temperature-dependent molecular rearrangement that results in the production of vitamin D[4]

This thermally labile product can isomerize to two biologically inert products, luminosterol and tachysterol. This alternative

photoisomerization prevents production of excessive amounts of vitamin D with prolonged sun exposure. The degree of skin pigmentation, which increases in response to solar exposure, also regulates the conversion of 7-dehydrocholesterol to vitamin D by blocking the penetration of ultraviolet rays.

Vitamin D is a hormone, not a vitamin-justification[46]

1. Vitamin D is synthesized in the skin by ultra violet rays of sunlight.
2. The biologically active form of vitamin D, calcitriol is produced in the kidney.
3. Calcitriol acts on specific target organs-intestine, bone and kidney.
4. Calcitriol action is similar to steroid hormones. It binds to a receptor in the cytosol and the complex acts on DNA to stimulate the synthesis of calcium binding protein.
5. Calcitriol synthesis is self regulated by a feedback mechanism. Calcitriol decreases its own synthesis.

Dietary sources of vitamin D[(5)]

1. Fortified dairy products
2. Egg yolks
3. Fish oils
4. Fortified cereal products.

Recommended dietary allowance (RDA)

The daily requirement of vitamin D is 400 international units or 10 mg of cholecalciferol. In countries with good sunlight like India, the RDA for vitamin D is 200 IU or 5 mg of cholecalciferol.

Vitamin D provided by plant sources is in the form of vitamin D_2, whereas that provided by animal sources is in the form of vitamin D_3. These two forms have equivalent biologic potencies and are activated equally efficiently by the hydroxylases in humans.

Absorption of vitamin D

Vitamin D is absorbed into the lymphatics and enters the circulation bound primarily to vitamin D binding protein, although a fraction of vitamin D circulates bound to albumin. The human vitamin D binding protein is an $alpha_2$–globulin, with a molecular mass of

approximately 52KD that is synthesized in the liver. The protein has a high affinity for 25(OH)D but also binds vitamin D and 1,25$(OH)_2$ D. Approximately 88% of 25(OH)D circulates bound to the vitamin D binding protein, 0.03% is free, and the rest circulates bound to albumin.[6] In contrast, 85% of the circulating 1,25$(OH)_2$ D_3 binds to the vitamin D binding protein, 0.4% is free, and the rest binds to albumin.[7] Thus, the role of vitamin D binding protein is to maintain a serum reservoir and to modulate the activity of vitamin D metabolites.

In the liver, vitamin D undergoes 25-hydroxylation by a cytochrome P450 like enzyme present in the mitochondria and microsomes. The half-life of 25(OH)D is approximately 2 to 3 weeks. The 25-hydroxylation of vitamin D is not tightly regulated. Therefore, the blood levels of 25(OH)D reflect the amount of vitamin D entering the circulation. When levels of vitamin D binding protein are low, such as in nephrotic syndrome, circulating levels of 25(OH)D are also reduced[8].

The half-life of 25(OH)D is shortened by increases in levels of its active metabolite, 1,25$(OH)_2D_3$ .The final step in the production of the active hormone is the renal 1α-hydroxylation of 25(OH)D to 1,25$(OH)_2D_3$. The half-life of this hormone is approximately 6 to 8 hours.Like the 25-hydroxylase,the1α-hydroxylase in the renal proximal convoluted tubule is a cytochrome P450 like mixed-function oxidase.[9,10,11] Unlike the 25-

hydroxylase, however, 1α-hydroxylase is tightly regulated. PTH and hypophosphatemia are the major inducers of this microsomal enzyme,[12] whereas calcium and the enzyme's product, $1,25(OH)_2$ D, repress it.

Hormones such as estrogen, calcitonin, growth hormone, and prolactin increase 1α-hydroxylase activity. However, the clinical importance of these observations has not been established. Ketoconazole has been shown to decrease levels of $1,25(OH)_2$ D_3 in a dose-dependent manner, presumably by interfering with 1α-hydroxylase activity.[16] 1α-Hydroxylase is also found in keratinocytes,[11] the trophoblastic layer of the placenta,[14] some lymphomas,[15] and granulomata, including sarcoid granulomata.[16]

In malignant and granulomatous tissue, the 1α-hydroxylase gene that is expressed is identical to that expressed in the kidney, but the gene is not regulated by PTH, phosphate, calcium, or vitamin D metabolites in these cells. Activation of macrophages with interferon, however, increases the expression of the 1-hydroxylase,[17] whereas treatment of sarcoidosis-associated hypercalcemia with glucocorticoids, ketoconazole,[18] or chloroquine[19] lowers serum $1,25(OH)_2$ D_3 levels.

The presence of 1α-hydroxylase in many target cells indicates autocrine and paracrine functions for $1,25(OH)_2D_3$ in the control of cell proliferation and differentiation. This local production of $1,25(OH)_2D_3$ is

dependent on circulating precursor levels, providing a potential explanation for the association of vitamin D deficiency with various cancers and autoimmune diseases.

1,25$(OH)_2D_3$ Metabolism

The high potency of 1,25$(OH)_2D_3$ in elevating serum calcium and phosphate levels requires a mechanism to attenuate its activity. This is accomplished within virtually all target cells by the 1,25$(OH)_2D_3$-inducible vitamin D 24-hydroxylase, which catalyzes a series of oxidation reactions at carbons 24 and 23, leading to side chain cleavage and inactivation. 24-Hydroxylase is regulated in a reciprocal manner to 1α-hydroxylase. Its activity and expression are increased by phosphate and reduced by PTH[20,21]. The 24-hydroxylase gene contains at least two distinct vitamin D response elements that mediate the effects of 1,25$(OH)_2D_3$ via its receptor on transcription[22,23].

1,25$(OH)_2D_3$ can also be converted to the 1,25(*R*)-$(OH)_2D_3$-23(*S*),26-lactone.[24] This metabolite has mild antagonist activity toward 1,25$(OH)_2D_3$ action[25] and more potent lactone analogues have now been developed. Recent studies have demonstrated that the 3β-hydroxyl group of 1,25$(OH)_2D_3$ can be epimerized to the 3α position[26,27] in a cell-specific

manner. 1, 25$(OH)_2$–3-epi-D_3 appears to be catabolised more slowly than the parent hormone and retains significant biological activity.

The significance of the 3-epimerase is not clear, but its cell-specific expression suggests that this pathway may function to prolong the activity of 1,25$(OH)_2D_3$ in cells containing this enzyme. Differential rates of 3-epimerization of vitamin D analogues may provide a mechanism for their selective actions in vivo. In addition, evidence suggests that 3-epimerization of select vitamin D analogues may enhance their proapoptotic activity. [28]

Transport Of Vitamin D

Vitamin D metabolites are lipophilic molecules with low aqueous solubility that must be transported in the circulation bound to plasma proteins. The most important of these carrier proteins is the vitamin D binding protein (DBP), which binds the metabolites with high affinity in the order 25(OH)D = 24,25$(OH)_2$D > 1,25$(OH)_2$D > vitamin D[(29)] .

Plasma levels of DBP are 20 times higher than the total amount of vitamin D metabolites, and >99% of circulating vitamin D compounds are protein bound, mostly to DBP, although albumin and lipoproteins contribute to lesser degrees. This has a major impact on their pharmacokinetics. DBP-bound vitamin D metabolites have limited access

to target cells[29] and, therefore, are less susceptible to hepatic metabolism and subsequent biliary excretion, leading to a longer circulating half-life.

Early evidence suggested that only the small fraction of unbound metabolites passively entered target cells to be further metabolized or to exert biological activity. For activated vitamin D compounds $1,25(OH)_2D_3$ and its analogues, biological activity was correlated with the concentration of free hormone. Thus DBP appears to buffer the free levels of active vitamin D compounds, guarding against vitamin D intoxication.

DBP levels are not regulated by vitamin D but are reduced by liver disease, nephrotic syndrome, and malnutrition and increased during pregnancy and estrogen therapy. The concentration of free $1,25(OH)_2D_3$, however, remains constant when DBP levels change, an example of the tight self-regulation of vitamin D metabolism.

ACTIONS OF VITAMIN D

Vitamin D Receptors

1,25-Dihydroxyvitamin D_3 exerts its biologic functions by binding to a nuclear receptor[30] which regulates transcription of DNA into RNA. The vitamin D receptor most closely resembles the retinoic acid, triiodothyronine, and retinoid-X receptors. The affinity of the receptor

for $1,25(OH)_2D_3$ is approximately three times higher than that for other vitamin D metabolites. Although $25(OH)D_3$ is less potent on a molar basis, its concentration in the serum is approximately three times higher than that of $1,25(OH)_2\ D_3$. However its free concentration is only twice that of $1,25(OH)_2D_3$. Therefore, under normal circumstances it is unlikely that $25(OH)D_3$ contributes importantly to calcium homeostasis.

Because the affinity of the vitamin D binding protein for 25(OH)D is greater than for $1,25(OH)_2\ D_3$, in states of vitamin D intoxication, the free levels of $1,25(OH)_2\ D_3$ increase [31] because 25(OH)D displaces it from the vitamin D binding protein. 25-OH D, therefore, may play a role in the clinical syndrome of vitamin D intoxication both by its direct biologic effects, when present at toxic levels, and by increasing free levels of $1,25(OH)_2\ D_3$.

Role of vitamin D on Intestinal Calcium Absorption

Under normal dietary conditions, calcium intake is in the range of 700 to 900 mg daily. Approximately 30% to 35% of this calcium is absorbed.[32] Absorption of calcium is impaired by bile salt deficiency, unabsorbed free fatty acids in steatorrheic states, and high dietary content of fibre or phytate. Gastric acid is needed to promote dissociation of calcium from anionic components of food or therapeutic preparations of calcium salts. Administration of calcium salts with meals, especially in

patients with achlorhydria, and use of divided doses or more soluble salts, such as calcium citrate, are commonly employed strategies to increase calcium bioavailability.

Calcium is thought to be absorbed by three pathways:

1. Transcellular route,
2. Vesicular calcium transport, and
3. Paracellular transport.

The first pathway is dependent on $1,25(OH)_2D_3$. Although the necessity of vitamin D for paracellular and vesicular calcium absorption remains controversial, substantial evidence exists that the hormone enhances this pathway as well[33,34].

Transcellular route

This pathway is thought to involve three steps:

1. Entry of calcium into the enterocyte,
2. Transport across the cell, and
3. Extrusion across the basolateral membrane.

Entry into the Enterocyte

A number of brush border proteins, including the intestinal membrane calcium-binding protein, brush border alkaline phosphatase,

and low-affinity Ca2+ -Mg2+ -ATPase, are induced by $1,25(OH)_2$ D_3. The activity of these proteins correlates with active calcium transport[35] .Two calcium channels, CaT1[36] and EcaC,[37] with six membrane-spanning domains are expressed in the duodenum and jejunum and have been implicated as important regulators of calcium entry into the enterocyte. Upon entering the enterocyte, calcium binds to components of the brush border complex subjacent to the plasma membrane. Calmodulin is redistributed to the brush border in response to $1,25(OH)_2$ D_3

Transport across cell

The best-studied effect of vitamin D on the enterocyte is the induction of synthesis of the intestinal calcium-binding protein calbindin 9K. This protein has an EF hand structure that permits the binding of two calcium ions per molecule. The affinity of calbindin for calcium is approximately four times that of the brush border calcium-binding components. Thus, calcium is preferentially transferred to calbindin. Calbindin serves to buffer the intracellular free calcium concentration during calcium absorption. It associates with microtubules and may play a role in the transport of calcium across the enterocyte. Organelles such as the mitochondria, Golgi, and endoplasmic reticulum also serve as repositories for intracellular calcium.

Exit from the Enterocyte

The transport of calcium across the antiluminal surface of the enterocyte, the final process involved in intestinal calcium absorption, is dependent on $1,25(OH)_2 D_3$. The main mechanism of calcium extrusion is the $1,25(OH)_2 D_3$ –inducible ATP-dependent Ca2+ pump. The affinity of the pump for calcium is approximately 2.5 times that of calbindin[38]. With high calcium intake, a $1,25(OH)_2 D_3$ -independent Na+ -Ca2+ exchanger may play a role in the transfer of calcium across the basolateral membrane as well.

Vitamin D-Actions on Bone

1,25-Dihydroxyvitamin D3 is a major transcriptional regulator of the two most abundant bone matrix proteins. It represses the synthesis of type I collagen[39] and induces the synthesis of osteocalcin[40]. 1,25-dihydroxyvitamin D3 promotes the differentiation of osteoclasts from monocyte-macrophage stem cell precursors in vitro, and it also increases osteoclastic bone resorption in high doses in vivo, by stimulating production of osteoclast-differentiating factor by osteoblasts.[41]

Vitamin D-Actions on kidney

Calcitriol is also involved in minimizing the excretion of calcium and phosphate through the kidneys, by decreasing their excretion and

enhancing reabsorption. All these effects ultimately lead to increase in plasma calcium level.

Vitamin D-Action on muscle

One of the striking clinical features of profound vitamin D deficiency that remains unexplained is the severe proximal myopathy [(42)]. Muscle cells express vitamin D receptors, and $1,25(OH)_2$ D_3 has nongenomic effects on muscle[(43)]. Furthermore, $1,25(OH)_2D_3$ increases amino acid uptake and alters phospholipid metabolism in vitro in muscle cells[(42)].

Vitamin D administration has been shown to increase the concentration of troponin C, a calcium-binding protein that plays a role in excitation coupling and increases the rate of uptake of calcium by the sarcoplasmic reticulum[(43)].

Vitamin D deficiency causes[(45)]

- Malabsorption, Dietetary deficiency, Iimpaired cutaneous production.
- Accelerated loss-Impaired enterohepatic circulation, Increased metabolism (Barbiturates, Phenytoin, Rifampin).
- Impaired 25 hydroxylation-Liver diseases, Isoniazid.

- Impaired 1 alpha hydroxylation-CKD, Hypoparathyroidism, 1-alpha hydroxylase mutation, Oncogenic osteomalacia, x-linked hypophosphatemic rickets.
- Target organ resistance -Phenytoin, vitamin D receptor mutation.
- Drugs-Phenytoin, INH, Barbiturates, Ketoconazole.

Vitamin D deficiency-Clinical features[46]

- RICKETS

Rickets is derived from an old English word wrick ken, meaning to twist. In childrens vitamin D deficiency results in growth retardation associated with an expansion of the growth plate. There will be expansion of the hypertrophic chondrocyte layer. It is characterized by bone deformities, resulting soft and pliable bones and delay in teeth formation. The weight-bearing bones are bent to form bow-legs.

- OSTEOMALACIA

Osteomalacia is derived from greek (osteon-bone; malakia-softness). There is impaired mineralization of bone matrix proteins , increasing their susceptibility to fracture.

- PROXIMAL MYOPATHY

The myopathy that accompanies vitamin D deficiency is characterized by normal creatine kinase levels, a myopathic electromyogram, and biopsy findings of loss of myofibrils, fatty infiltration, and interstitial fibrosis. The myopathy resolves within days to weeks of vitamin D replacement and is not related to normalization of mineral ion homeostasis.

Vitamin D deficiency – Laboratory Diagnosis[45]

1) Serum 25(OH)D level

- optimal level in serum >25 ng/ml
- in vitamin D deficiency, it will be less than 15 ng/ml
- paradoxically $1,25(OH)_2$ D are often normal in severe vitamin D deficiency. Since, PTH is major stimuli for 1 alpha hydroxylase, there is increased level of $1,25(OH)_2D$

2) Low serum calcium level

3) Low serum phosphorus level

4) Elevated serum alkaline phosphatase level

5) Radiography

- widended , expanded growth plate

- swelling of costochondral junction, rachitic rosary
- decrease in cortical thickness and relative radiolucency of skeleton
- Pseudofracture or looser's zones

  Radiolucent lines that occurs in areas of contact with the underlying skeletal element

Vitamin D deficiency-Treatment[45]

- Vitamin D supplementation

  50,000 IU weekly for 3-12 weeks followed by 800 IU daily as maintenance dose

- Calcium supplementation

  1.5 to 2 g/day of elemental calcium

- Normocalcemia is observed within one week of therapy
- Elevated ALP and PTH may persist for 6 month

CALCIUM HOMEOSTASIS

Ca^{2+} is a universal intracellular messenger that participates in numerous biological processes from neural regulation to muscle contraction, and from gene expression to cell growth and death. It

is the most abundant among the minerals in the body. It is therefore no surprise there that a variety of mechanisms exist to ensure that plasma calcium concentrations are kept within a relatively narrow band.

In an average adult human, there is over 1 kg of calcium within the body. Most of this is present within the skeleton, where the vast majority is in the form of relatively insoluble calcium hydroxyapatite, although there is some more soluble complexed calcium. Further 0.6% of body's calcium is found within teeth, with a similar amount being found in the soft tissues and a much smaller quantity being present within the body fluids.

In plasma, calcium exists in three forms. The most important of these comprising approximately 50% of the total calcium, is its ionized form. This calcium is fully available for use within biological systems. This fraction of calcium is subject to physiological control. Further 5% of calcium is loosely complexed to anions such as citrate or phosphates. In this form calcium is not directly available for biological processes but can rapidly dissociate from the anions and become available. Third, plasma calcium (45%) is bound to plasma proteins, particularly albumin.

Biochemical functions of calcium[46]

1) Development of bones and teeth

2) Muscle contraction

Calcium interacts with troponin c to trigger muscle contraction, also increases interaction between actin and myosin

3) Blood coagulation

Calcium acts as factor 4 in coagulation pathway

4) Nerve transmission

5) Membrane integrity and permeability

Calcium influences the membrane structure and transport of water and several ions.

6) Activation of enzymes

Calcium is needed for the direct activation of enzymes such as lipase, ATPase and succinate dehydrogenase.

7) Calmodulin mediated action of calcium

Calcium-calmodulin complex activates certain enzymes like, adenylate cyclise, calcium dependent protein kinases.

8) Calcium is an intracellular messenger

Calcium is regarded as a second messenger for some hormonal

action like for epinephrine in liver glycogenolysis , and for ADH action on kidney.

9) Release of hormones

Insulin, PTH, calcitonin release is facilitated by calcium

10) Secretory process

11) Role in cell-to-cell communication

12) Acts on myocardium and prolongs systole

Calcium- Normal ranges[46]

The serum level of calcium is closely regulated with a normal total calcium of 2.2-2.6 mmol/L (9-10.5 mg/dL) and a normal ionized calcium of 1.1-1.4 mmol/L (4.5-5.6 mg/dL). The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound. The biologic effect of calcium is determined by the amount of ionized calcium, rather than the total calcium. Ionized calcium does not vary with the albumin level, and therefore it is useful to measure the ionized calcium level when the serum albumin is not within normal ranges.

Corrected calcium level[47]

One can derive a corrected calcium level when the albumin is abnormal. This is to make up for the change in total calcium due to the

change in albumin-bound calcium, and gives an estimate of what the calcium level would be if the albumin were within normal ranges.

Corrected calcium (mg/dL) = Measured total Ca (mg/dL) +

0.8 (4.0 - Serum albumin [g/dL]),

4.0 represents the average albumin level in g/dL.

Every 1 g/dL decrease of albumin will decrease 0.8 mg/dL in measured serum calcium and thus 0.8 must be added to the measured calcium to get a corrected calcium value.

Sources of calcium

- Milk
- Egg
- Fish
- Vegetables
- Wheat
- Rice

Factors necessary for Absorption

1. Calcium absorption is increased by the following:

a. Vitamin D

b. Parathyroid hormone

c. Acidity

d. Aminoacids

2. Calcium absorption is decreased by the following:

a. Phytic acid in cereals

b. Oxalates in leafy vegetables

c. Malabsorption syndrome

d. High phosphate content

Low Plasma Calcium: Stimulates PTH release, and PTH acts to reabsorb Ca^{2+} from the pool in bone and to enhance renal re-absorption of Ca^{2+}

High Plasma Calcium: Stimulates calcitonin CT secretion which lowers plasma calcium by inhibiting bone resorption.

HYPOCALCEMIA(serum calcium level<8.5 mg/dl)[46]

Hypocalcemia can present as an asymptomatic laboratory finding or as a severe, life-threatening condition .In the setting of acute hypocalcemia, rapid treatment may be necessary. In contrast, chronic hypocalcemia may be well tolerated, but treatment is necessary to prevent long-term complications.

HYPOCALCEMIA-CLINICAL FEATURES[63]

Signs and symptoms due to Neuromuscular irritability

- Chvostek's sign
- Trousseau's sign
- Paresthesias
- Tetany
- Seizures (focal, petit mal, grand mal)
- Fatigue
- Anxiety
- Muscle cramps
- Polymyositis
- Laryngeal spasms, Bronchial spasms

Other Neurological signs

- Extrapyramidal signs due to calcification of basal ganglia
- Calcification of cerebral cortex or cerebellum
- Personality disturbances
- Irritability
- Impaired intellectual ability
- Nonspecific EEG changes
- Increased intracranial pressure
- Parkinsonism
- Choreoathetosis

Ectodermal changes

- Dry skin
- Coarse hair
- Brittle nails
- Alopecia
- Enamel hypoplasia
- Shortened premolar roots
- Thickened lamina dura
- Delayed tooth eruption
- Increased dental caries
- Atopic eczema
- Exfoliative dermatitis

Smooth muscle involvement

- Dysphagia
- Abdominal pain
- Biliary colic
- Dyspnea

Ophthalmologic manifestations

- Subcapsular cataracts
- Papilledema

Cardiac manifestations

- Prolonged QT interval in ECG
- Congestive heart failure and cardiomyopathy

The hallmark of acute hypocalcemia is neuromuscular irritability. Clinically, neuromuscular irritability can be demonstrated by eliciting Chvostek's or Trousseau's signs.

Chvostek's sign (Weiss sign)

It was first described by Frantichek chvostek in 1876[48]

CAUSES:

1. Hypocalcemia
2. Hypomagnesemia
3. Respiratory alkalosis , particularly following hyperventilation

Tapping the skin over the facial nerve anterior to the external auditory meatus produces this sign. Ipsilateral contraction of the facial muscles occur due to hyperexcitability of facial nerve.

Trousseau's sign

Trousseau’s sign is thought to be both sensitive and specific for hypocalcemic tetany[49,50]. This sign is induced by inflation of a blood pressure cuff to 20 mm Hg above the patient's systolic blood pressure for 3-5 minutes. Carpal spasm presents as flexion of the wrist in metacarpo phalangeal joints, extension of the interphalangeal joints, and abduction of the thumb.

ANTICONVULSANTS AND CALCIUM HOMEOSTASIS[52,53,54,55]

Chronic usage of anticonvulsant therapy may interfere with calcium homeostasis in many ways as follows,

1) They can accelerate hepatic inactivation of vitamin D[56,57]

The anticonvulsant therapy is associated with induction of hepatic microsomal system resulting in increased catabolism and excretion of vitamin D and depletion of biologically active vitamin D metabolites in the body. In view of this observations one would expect that the decreased availability of 25(OH)D substrate would cause a deficiency of the active metabolite1, $25(OH)_2D$ leading to rickets and osteomalacia.

2) They can cause target organ resistance to vitamin D action[58,59]

3) They can directly reduces the calcium absorption from GIT[56,57]

Anticonvulsants may inhibit gastrointestinal calcium absorption and bone formation through end-organ hypo responsiveness to $1,25(OH)_2D$ and by reduced tissue concentration of active vitamin D metabolites

4) They impair PTH/1, $25(OH)_2D_3$ mediated bone resorption[60]

5) Inhibition of calcitonin secretion[60].

6) Inhibition of Calcium and phosphorus Excretion[61]

Antiepileptic drugs may provoke renal conservation of calcium and phosphate and possibly compensate partly the above mentioned negative effects on calcium and bone metabolism. This may explain the relatively low incidence of manifest rickets or osteomalacia despite long-term treatment with drugs that are now well known to interfere with so many important factors of calcium homeostasis.

Because of this hypocalcemia, the parathyroid glands are stimulated to produce PTH to maintain the serum calcium level. The parathormone tries to maintain the serum calcium level by its action on kidney, bone, and vitamin D mediated increased calcium absorption from GIT. Due to the PTH – induced increase in bone turn over, alkaline phosphatase levels are often elevated. Phenytoin[53], Phenobarbitone, Sodium Valproate, and Carbamazepine have been proven to cause disturbances in calcium homeostasis through one or more of the above mentioned mechanisms.[53,54,55]

SEIZURE CONTROL AND HYPOCALCEMIA[51]

If the patient has pre-existing subclinical epilepsy, hypocalcemia may lower the excitation threshold for seizures. Electroencephalographic changes may be acute and nonspecific or present with distinct changes in the electroencephalogram (EEG). EEG changes may be present with or without symptoms of hypocalcemia. Some patients on anticonvulsive therapy have not needed medication after appropriate treatment of their hypocalcemia. The relationship between calcification of basal ganglion, cerebral cortex, or cerebellum with pre-existing epileptic or convulsive disorders is not well understood. In the elderly population, disorientation or confusion may be manifestations of hypocalcemia.

ALKALINE PHOSPHATASE (ALP)

ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase[52].

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta.

The normal range is 20 to 180 IU/L.[63] Levels are significantly higher in children and pregnant women. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a by product of osteoblast activity.

When ALP is elevated, isoenzyme studies using electrophoresis can confirm the source of the ALP. Heat stability also distinguishes bone and liver isoenzymes.

Causes For Elevated Alkaline Phosphatase

Liver ALP

1) Cholestasis,
2) Cholecystitis,
3) Cholangitis,
4) Cirrhosis, hepatitis, fatty liver,
5) Sarcoidosis,
6) Liver tumor,
7) Liver metastasis
8) Drugs intoxication

Concurrently elevated GGT (gamma glutamyl transpeptitase) helps rule in favour of liver metastasis (rather than bone, kidney) when assessing spread of cancer.

Bone/skeletal causes

Bone diseases

1) Paget's disease, Osteosarcoma ,Secondaries

2) Renal osteodystrophy

3) Multiple myeloma

4) Bone fracture

Skeletal involvement of other primary diseases

1) Vitamin D deficiency

2) Osteomalacia

3) Rickets

4) Secondary hyperparathyroidism

Other causes

1) Polycythemia vera

2) Myelofibrosis

3) Leukemoid reaction due to infection

4) Pregnancy

5) Women using hormonal contraception

PARATHYROID HORMONE (PTH)

Parathormone or parathyrin, is secreted by the parathyroid glands as a polypeptide containing 84 amino acids. PTH acts to increase the concentration of calcium in the blood by acting upon parathyroid receptors in three parts of the body. PTH half-life is approximately 4 minutes.(64) It has a molecular mass of 9.4 KDa.[65]

PTH-Action on bone

It enhances the release of calcium from the large reservoir contained in the bones.[66] Bone resorption is the normal destruction of bone by osteoclasts, which are indirectly stimulated by PTH. Stimulation is indirect, since osteoclasts do not have a receptor for PTH. PTH binds to osteoblasts, the cells responsible for creating bone. Binding stimulates osteoblasts to increase their expression of RANKL and inhibits their expression of osteoprotegrin. It binds to RANKL and blocks it from interacting with RANK, a receptor for RANKL. The binding of RANKL to RANK stimulates these osteoclast precursors to fuse, forming new osteoclasts which ultimately enhance bone resorption.

PTH-Action on kidney

It enhances active reabsorption of calcium and magnesium from distal tubules and the thick ascending limb. As bone is degraded both calcium and phosphate are released. It also greatly increases the excretion of phosphate, with a net loss in plasma phosphate concentration. It thus increases the calcium:phosphate ratio and more calcium is free in circulation.

PTH-Action on Gut

It enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D. Vitamin D activation occurs in the kidney. PTH up-regulates 25-hydroxyvitamin D_3 1-alpha-hydroxylase, the enzyme responsible for 1-alpha hydroxylation of 25 hydroxy vitamin D, converting vitamin D to its active form (1,25-dihydroxy vitamin D). This activated form of vitamin D increases the absorption of calcium (as Ca^{2+} ions) by the intestine via calbindin.

Regulation of PTH secretion

Secretion of parathyroid hormone is controlled chiefly by serum [Ca^{2+}] through negative feedback. Calcium sensing receptors located on parathyroid cells, are activated when [Ca^{2+}] is low[67] . In the

parathyroid gland, sensation of high concentrations of extracellular calcium result in activation of the Gq G-protein coupled cascade through the action of phospholipase C. This hydrolyzes phosphatidylinositol 4,5-bisphosphae (PIP_2) to liberate intracellular messengers IP_3 and diacylglycerol (DAG). Ultimately, these two messengers result in a release of calcium from intracellular stores and a subsequent flux of extracellular calcium into the cytoplasmic space. The effect of this signaling of high extracellular calcium results in an intracellular calcium concentration which inhibits the secretion of preformed PTH from storage granules in the parathyroid gland.

Stimulators of PTH

1) Decreased serum [Ca^{2+}].

2) Mild decreases in serum [Mg^{2+}].

3) An increase in serum phosphate

Since increased phosphate will complex with serum calcium to form calcium phosphate, which causes the Ca-sensitive receptors (CaSR) to think that serum Ca has decreased, as CaSR do not sense Calcium phosphate, thereby triggering an increase in PTH.

Inhibitors of PTH

1) Increased serum [Ca^{2+}]

2) Severe decreases in serum [Mg^{2+}], which also produces symptoms of hypoparathyroidism.

Measurement

PTH can be measured in the blood in several different forms intact PTH N-terminal PTH, mid-molecule PTH, and C-terminal PTH, and different tests are used in different clinical situations. The average PTH level is 10-80 pg/ml[(42)].

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN

Cross sectional single centred prevalence study

STUDY CENTRE

Government General Hospital, Chennai

Period of the study : May 2010 to October 2010

Ethical clearance : Granted

Consent : Informed consent from all patients.

INCLUSION CRITERIA

1) Patients attending the epilepsy clinic who are on the following single or multiple anti- convulsants commonly used in our hospital,
   - Phenytoin
   - Phenobarbitone
   - Sodium valproate
   - Carbamazepine
2) Patients taking the above mentioned drugs for more than 6 months
3) Any patient with seizure disorder was considered, irrespective of whether the seizures were primary or secondary

EXCLUSION CRITERIA

1) Patients with chronic kidney disease

2) Patients with chronic liver disease

3) Malabsorption syndromes

The above mentioned conditions may per se cause disturbances in calcium homeostasis and hence were excluded from the study.

4) Patients on any other drug that may interfere with calcium homeostasis

5) Non compliance with anti convulsant therapy

ANALYSIS

Data analyzed using statistical package - SPSS Software.

CONFLICT OF INTEREST - Nil.

EXTERNAL FINANCIAL SUPPORT – Nil

METHODOLOGY

- 100 patients with seizure disorder attending the GGH, Chennai, were randomly selected for the study, after applying the inclusion and exclusion criteria.
- Detailed history regarding epilepsy treatment, compliance and seizure control was taken from all the patients enrolled for the study.

- The data regarding the number and duration of anti convulsant usage was collected
- Complete physical examination was carried out on all the patients.
- Serum calcium, vitamin D, Alkaline phosphatase and Parathormone levels were measured.
- Calcium levels were corrected in cases with hypoalbuminemia.
- Renal and Liver function tests were done to rule out other important causes of hypovitaminosis D.
- The results were analysed using SPSS software statistical pack.

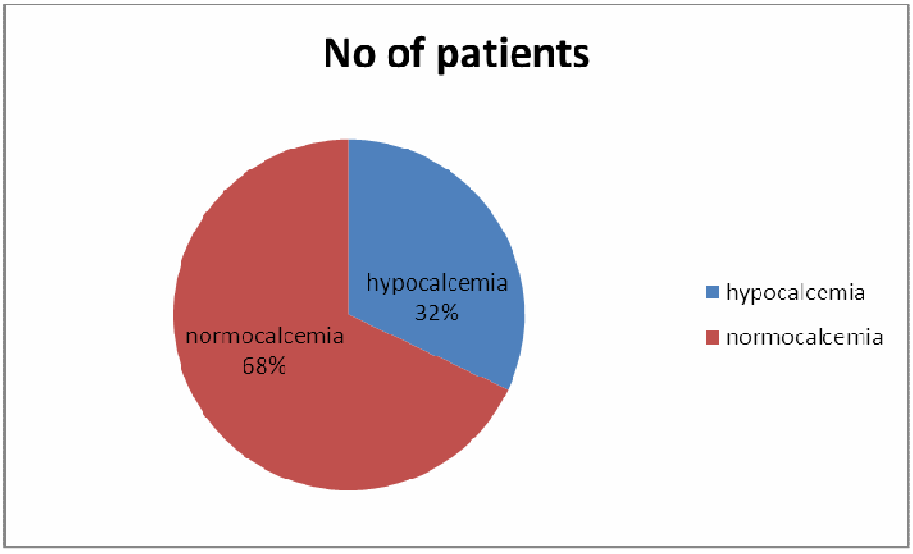
RESULTS AND OBSERVATION

RESULTS AND OBSERVATION

TABLE - 1

Prevalence of hypocalcemia in patients on chronic Anticonvulsant Therapy

Calcium level	No of patients	Percentage
Hypocalcemia	32	32%
Normocalcemia	68	68%
Total	100	100%

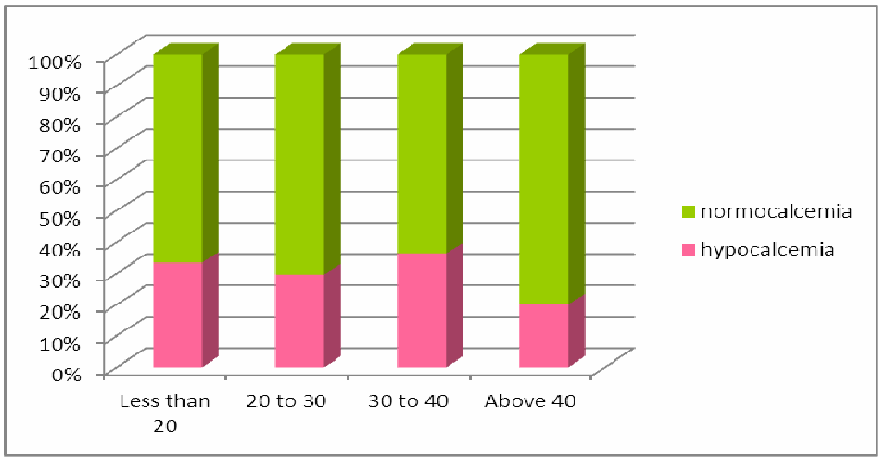


The prevalence of hypocalcemia in patients taking anticonvulsants for a long time (>6 months) is 32%.

TABLE - 2

Age distribution and hypocalcemia

Age Group in years		Normocalcemia	Hypocalcemia	Total	P value
Below 20	Number	20	10	30	>0.005
	percentage	66.7%	33.3%	100.0%	
21-30	Number	19	8	27	
	percentage	70.4%	29.6%	100.0%	
31-40	Number	21	12	33	
	percentage	63.6%	36.4%	100.0%	
Above 40	Number	8	2	10	
	percentage	80.0%	20.0%	100.0%	
Total	Number	68	32	100	
	percentage	68.0%	32.0%	100.0%	

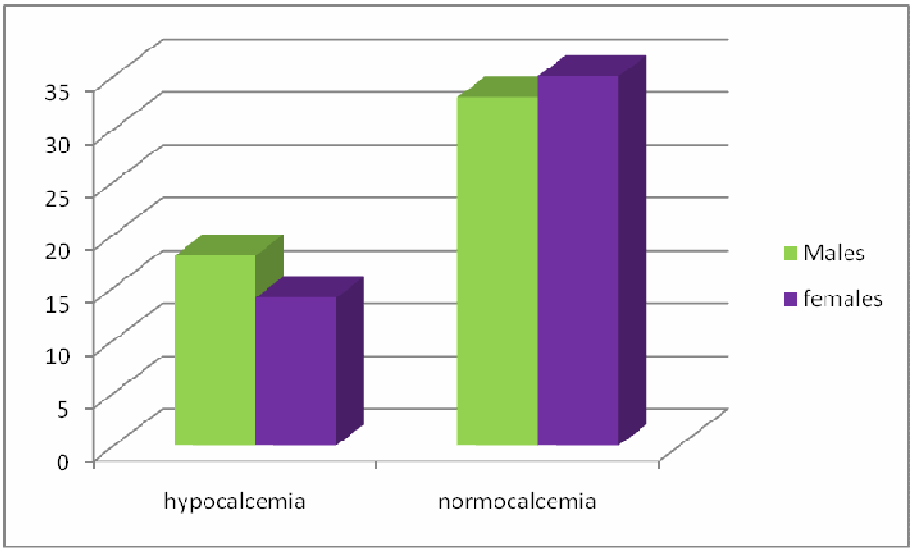


There is no significant correlation between age and incidence of hypocalcemia in patients taking chronic anticonvulsant therapy

TABLE - 3

Sex distribution and hypocalcemia

Gender		Hypocalcemia	Normocalcemia	Total	Significance
Males	Count	18	32	50	>0.005
	Percentage	36.0%	64.0%		
Females	Count	14	36	50	
	percentage	28.0%	72.0%		
Total	count	32	68	100	
	percentage	32.0%	68.0%		

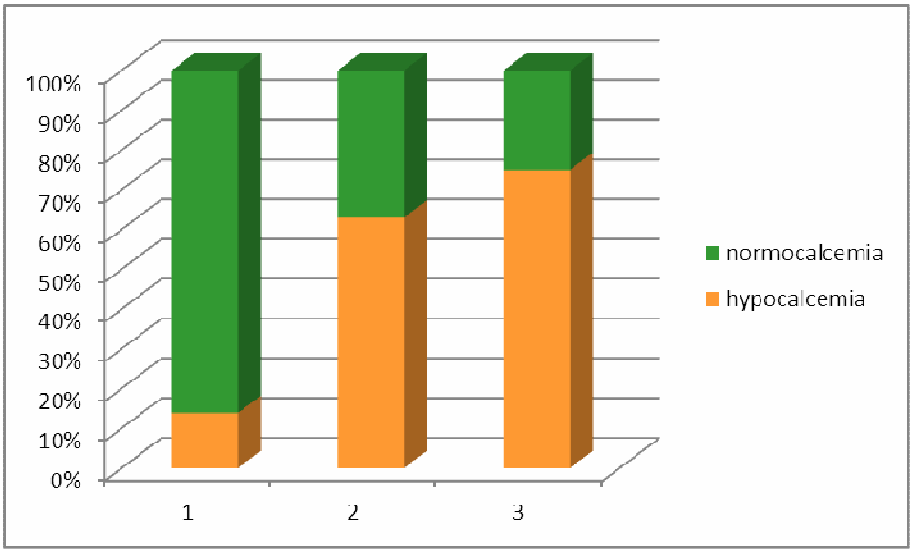


There is no significant association between the age distribution and incidence of hypocalcemia in patients on chronic anticonvulsant therapy.

TABLE - 4

Number of drugs and calcium level

No. of Drugs		Normocalcemia	Hypocalcemia	Total	P value
1	Number	56	9	65	<0.001
	percentage	86.2%	13.8%	100.0%	
2	Number	10	17	27	
	percentage	37.0%	63.0%	100.0%	
3	Number	2	6	8	
	percentage	25.0%	75.0%	100.0%	
Total	Number	68	32	100	
	percentage	68.0%	32.0%	100.0%	

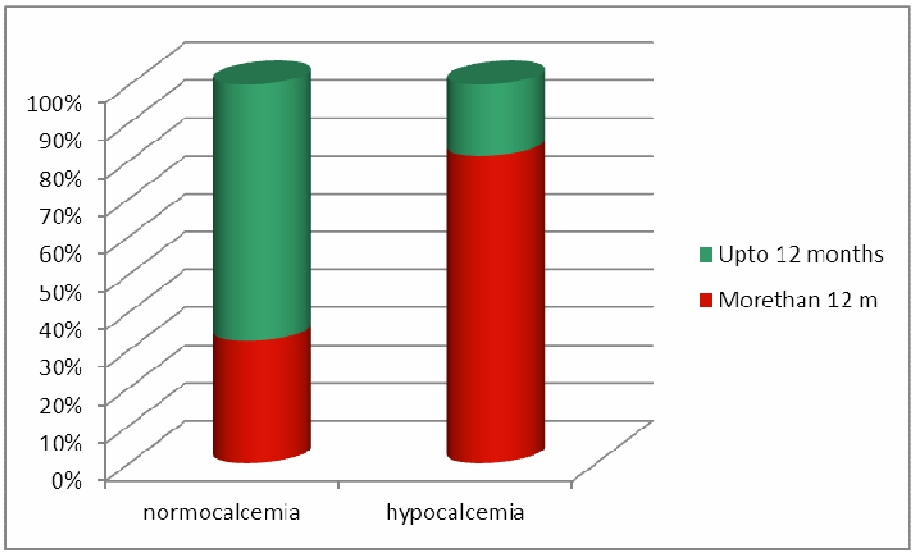


The prevalence of hypocalcemia is higher in patients using more number of anticonvulsant drugs.

TABLE - 5

Duration of treatment and calcium level

Duration		Hypocalcemia	Normocalcemia	Total	P value
Upto 12 months	Number	6	46	52	<0.001
	Percentage	11.5%	88.5%		
More than 12 months	Number	26	22	48	
	Percentage	54.2%	45.8%		
Total	Number	32	68	100	
	Percentage	32.0%	68.0%		

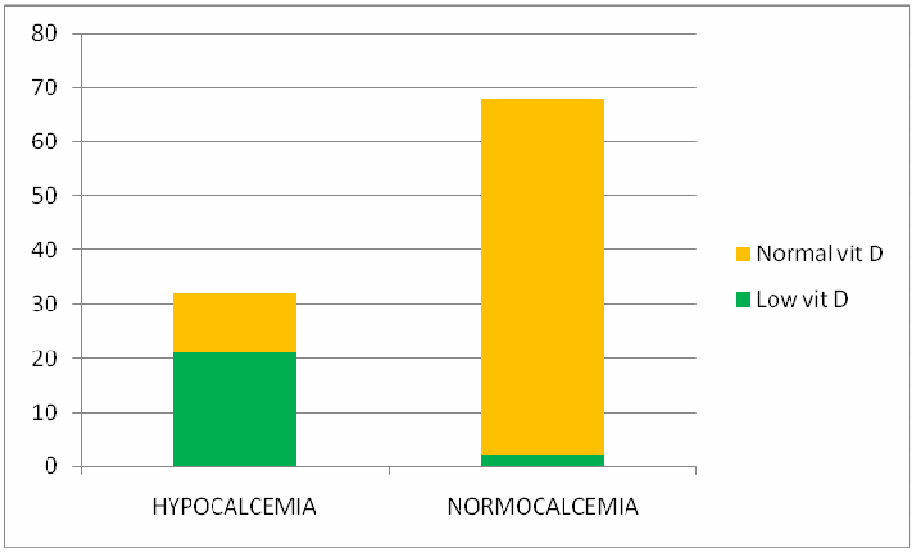


In patients taking anticonvulsants for more than a year, the chance of developing hypocalcemia is higher when compared to patients on drug treatment for less than a year.

TABLE -6

Correlation between hypocalcemia and low vitamin D

Vitamin D		Low calcium	Normal calcium	Total	P value
Low Vit D	Count	21	2	23	<0.001
	Percentage	91.3%	8.7%		
Normal Vit D	Count	11	66	77	
	Percentage	14.3%	85.7%		
Total	Count	32	68	100	
	percentage	32.0%	68.0%		

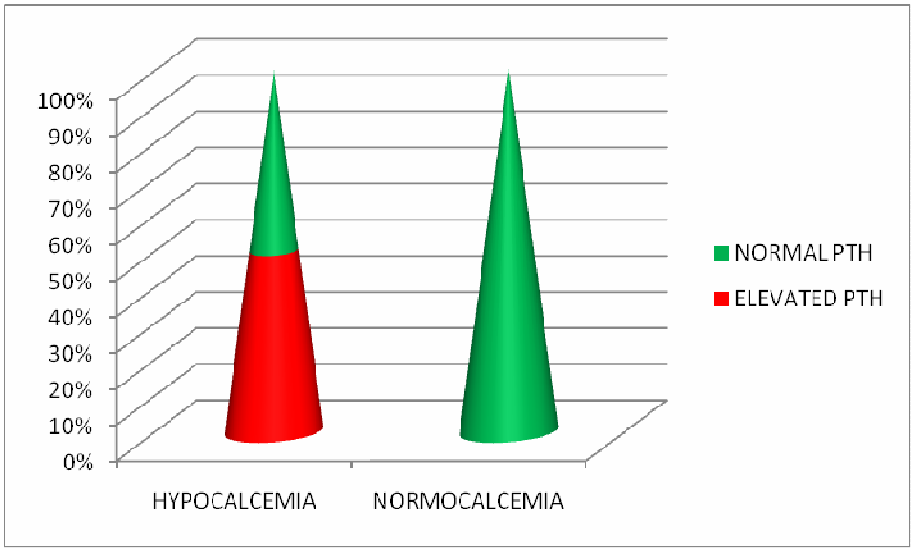


Presence of hypocalcemia was associated with low serum levels of vitamin D in a significant number of patients

TABLE - 7

Correlation between hypocalcemia and elevated PTH

PTH Level		Hypocalcemia	Normocalcemia	Total	P value
Elevated PTH	Number	16	0	16	<0.001
	Percentage	100%	0%		
Normal PTH	Number	16	68	84	
	Percentage	20.5%	80.9%		
Total	Number	32	68	100	
	percentage	32.0%	68.0%		

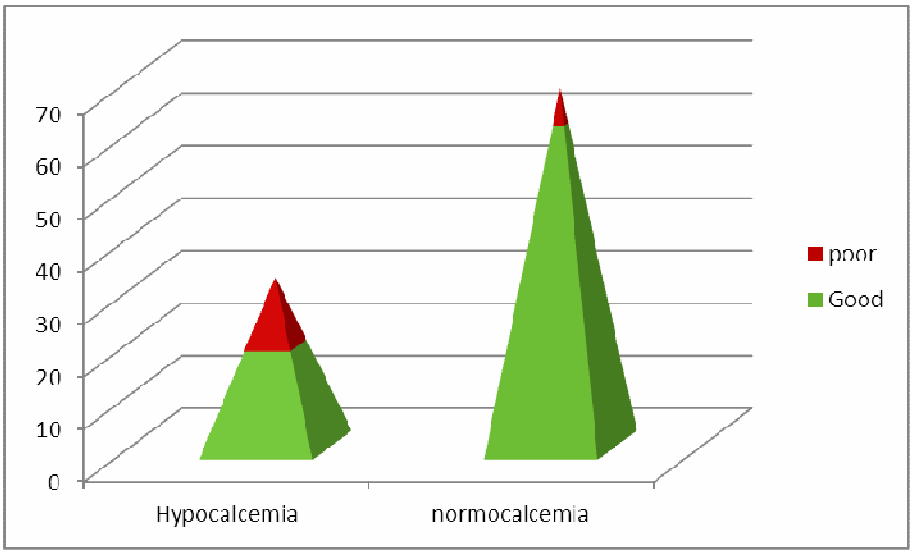


50% of hypocalcemic patients had secondary hyperparathyroidism.

TABLE - 8

Seizure control and calcium level

Seizure control		Hypocalcemia	Normocalcemia	Total	P value
Good	Number	19	61	80	< 0.001
	Percentage	23.8%	76.3%		
Poor	Number	13	7	20	
	Percentage	65.0%	35%		
Total	Number	32	68	100	
	Percentage	32.0%	68.0%		

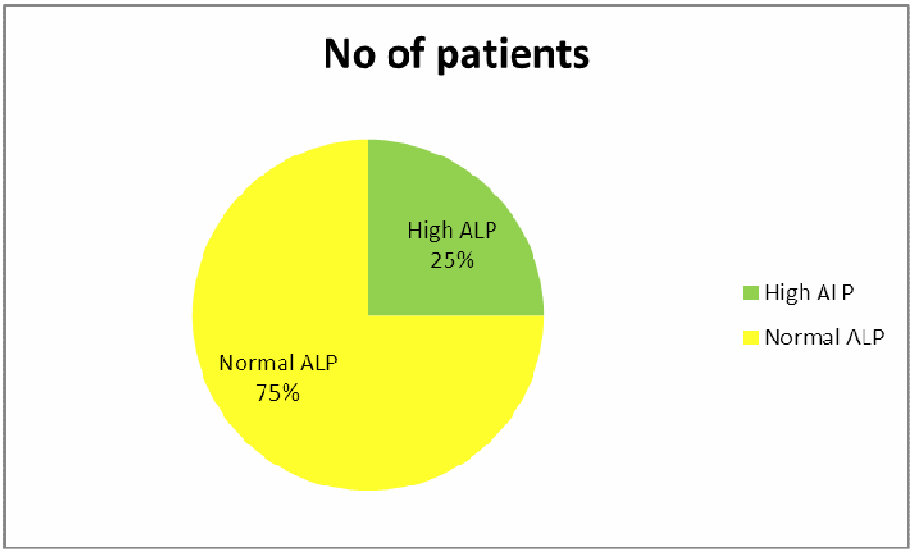


Hypocalcemia is associated with a higher incidence of poor seizure control .

TABLE - 9

Prevalence of abnormality in ALP in patients on chronic anticonvulsant therapy

ALP level	No of patients	Percentage
High ALP	25	25%
Normal ALP	75	75%
Total	100	100%

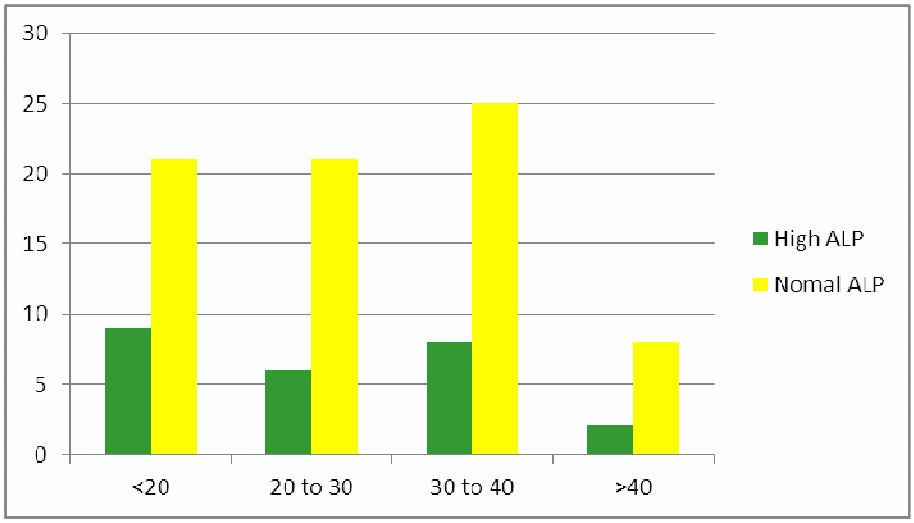


The prevalence of elevated alkaline phosphotase in patients taking anticonvulsants for a long time (>6 months) is 25%.

TABLE - 10

Age distribution and elevated ALP

Age		High ALP	Normal ALP	Total	P value
<20	Number	9	21	30	>0.005
	Percentage	30.0%	70.0%		
20 to 30	Number	6	21	27	
	Percentage	22.2%	77.8%		
30 to 40	Number	8	25	33	
	Percentage	24.2%	75.8%		
>40	Number	2	8	10	
	Percentage	20.0%	80.0%		
Total	Number	25	75	100	
	Percentage	25.0%	75.0%		

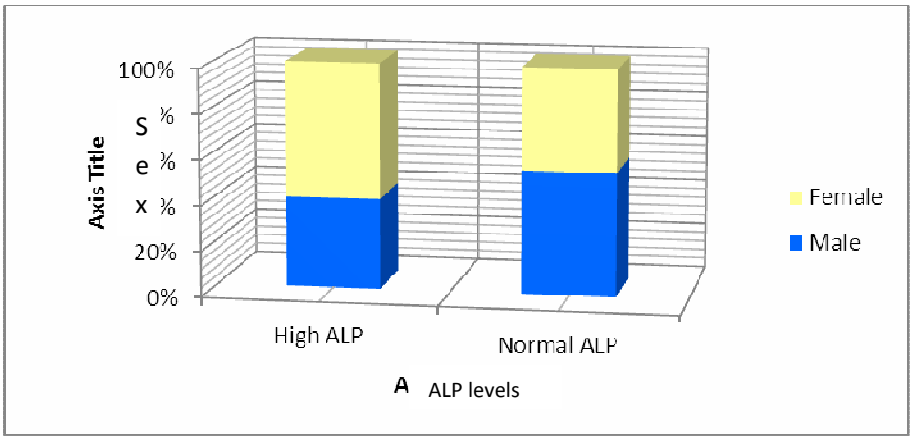


There is no significant relation between age distribution of the study population and the incidence of elevated ALP.

TABLE - 11

Sex distribution and elevated ALP

SEX		High ALP	Normal ALP	Total	P value
Male	Number	10	40	50	>0.005
	percentage	20.0%	80.0%		
Female	Number	15	35	50	
	percentage	30.0%	70.0%		
Total	Number	25	75	100	
	percentage	25.0%	75.0%		

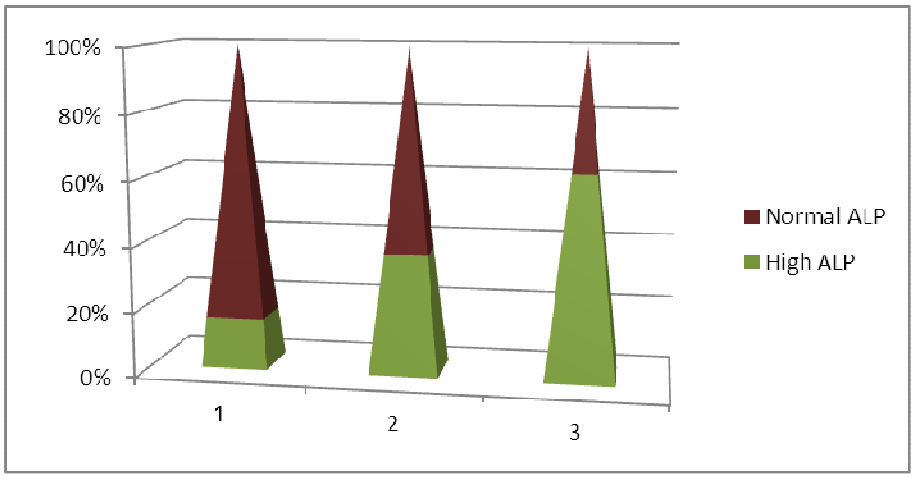


No difference in incidence of elevated ALP was found between both the sexes

TABLE - 12

Number of anticonvulsants and elevated ALP

No of anticonvulsants		High ALP	Normal ALP	Total	P value
1	Number	10	55	65	<0.001
	Percentage	15.4%	84.6%		
2	Number	10	17	27	
	Percentage	37.0%	63.0%		
3	Number	5	3	8	
	percentage	62.5%	37.5%		
Total	Number	25	75	100	
	percentage	25.0%	75.0%		

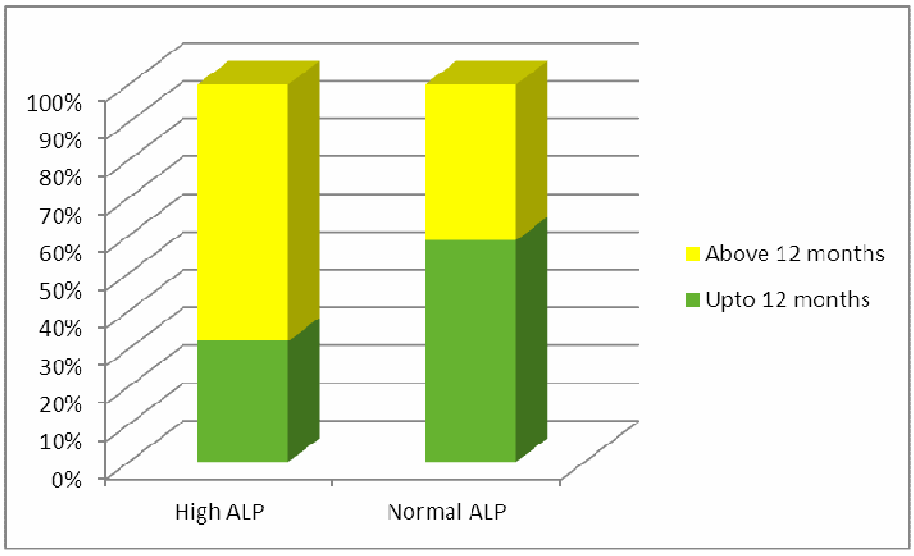


With increasing number of anti convulsants, there is an increased prevalence of elevaled ALP.

TABLE - 13

Duration of treatment and high ALP

Duration of treatment		High ALP	Normal ALP	Total	P value
Upto 12 months	Number	8	44	52	<0.001
	Percentage	15.4%	84.6%		
Above 12 months	Number	17	31	48	
	Percentage	35.4%	64.6%		
Total	Number	25	75	100	
	percentage	25.0%	75.0%		

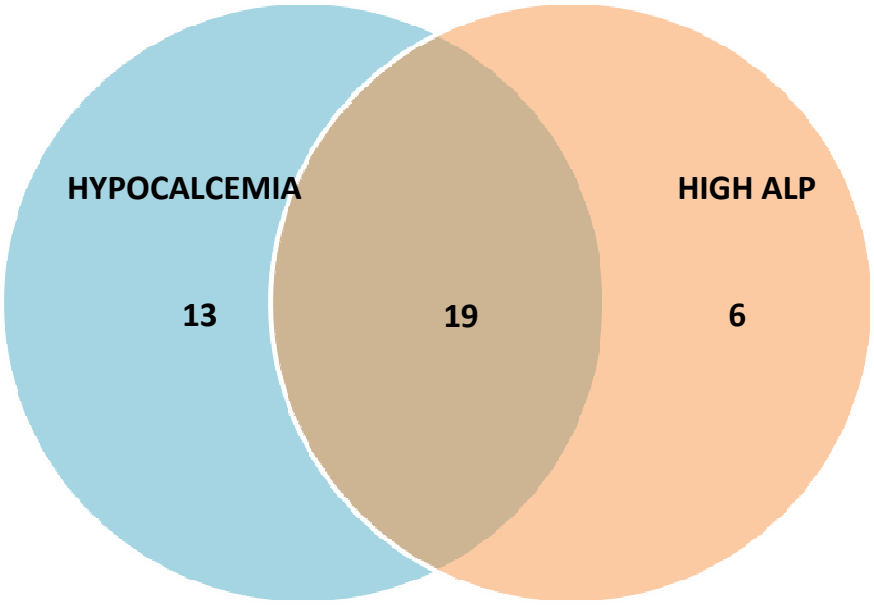


The longer the anti convulsant usage, higher is the chance of elevated ALP.

TABLE - 14

Relationship of hypocalcemia and high ALP

Calcium level		Normal ALP	High ALP	Total	P value
Normocalcemia	Number	62	6	68	<0.005
	Percentage	91.2%	8.8%		
Hypocalcemia	Number	13	19	32	
	Percentage	40.7%	59.3%		
Total	Number	75	25	100	
	Percentage	75.0%	25.0%		

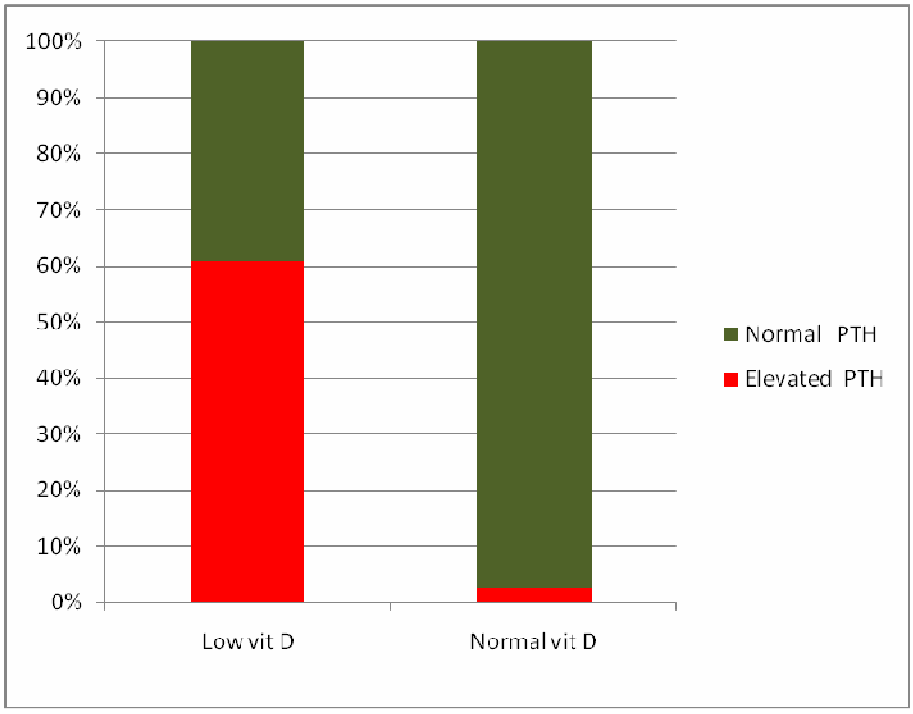


Hypocalcemia correlated with elevated ALP and normocalcemia with normal ALP levels.

TABLE -15

Correlation between vitamin D and PTH level

PTH level		Low Vit D	Normal Vit D	Total	P value
Elevated PTH	Number	14	2	16	<0.001
	Percentage	87.5%	12.5%		
Normal PTH	Number	9	75	84	
	Percentage	10.8%	89.2%		
Total	Number	23	77	100	
	percentage	23.0%	77.0%		

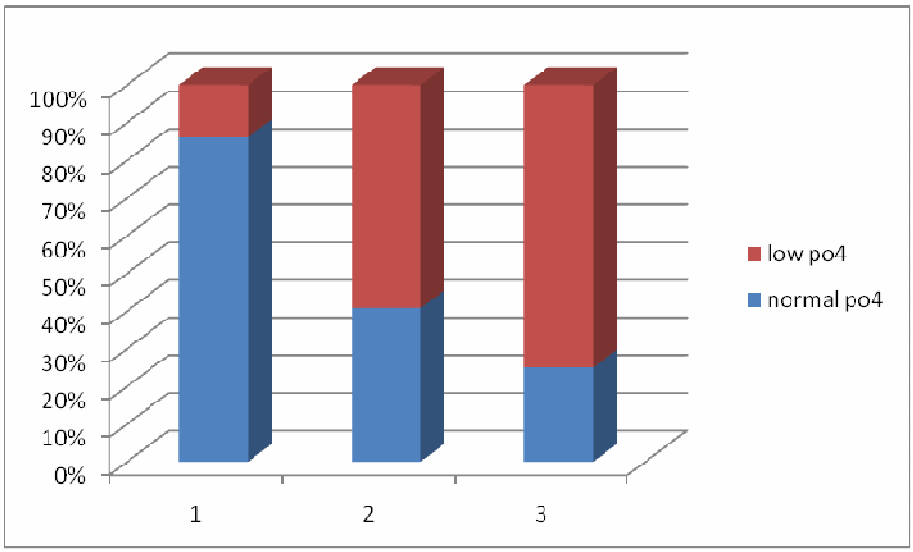


A significant number of patients with low vitamin D values also had elevated PTH levels.

TABLE -16

Correlation of serum phosphorus and number of anticonvulsants

Number of drugs		Hypophosphatemia	Normal PO_4^+	Total	P Value
1	Number	9	56	65	
	Percentage	13.8%	86.2%	100%	
2	Number	16	11	27	
	Percentage	59.3%	40.7%	100%	<0.001
3	Number	6	2	8	
	Percentage	75%	25%	100%	
Total		31	69	100	

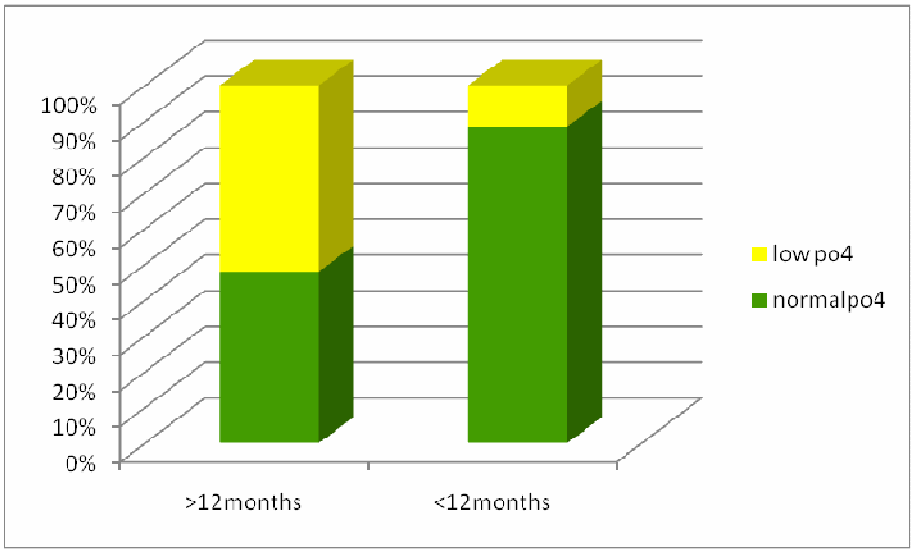


Increased number of anti convulsant drug usage was associated with a higher incidence of hypophosphatemia.

TABLE -17

Duration of anticonvulsants and phosphate level

Duration		Normal po_4	Low po_4	Total	P value
>12 months	Number	6	46	52	<0.001
	percentage	11.5%	88.5%	100%	
<12 months	Number	25	23	48	
	percentage	52.1%	47.9%	100%	
Total		31	69	100	

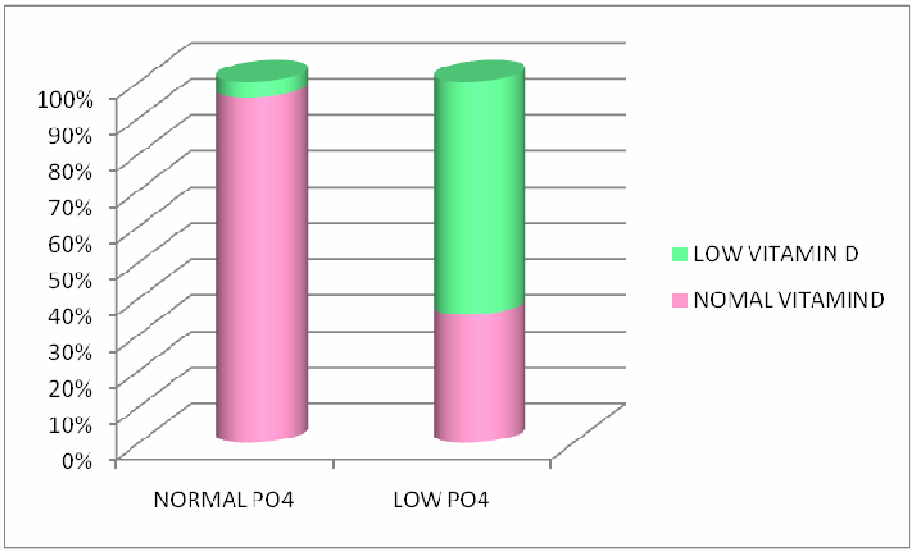


Prevalence of hypophosphatemia increases as the duration of drug treatment increases.

TABLE -18

Correlation of serum phosphorus and vitamin D

Vitamin D		Normal po_4	Low po_4	Total	P value
Normal	Number	66	11	77	<0.001
	Percentage	85.7%	14.3%	77.0%	
Low	Number	3	20	23	
	Percentage	13.0%	87.0%	23.0%	
Total		69	31	100.0%	

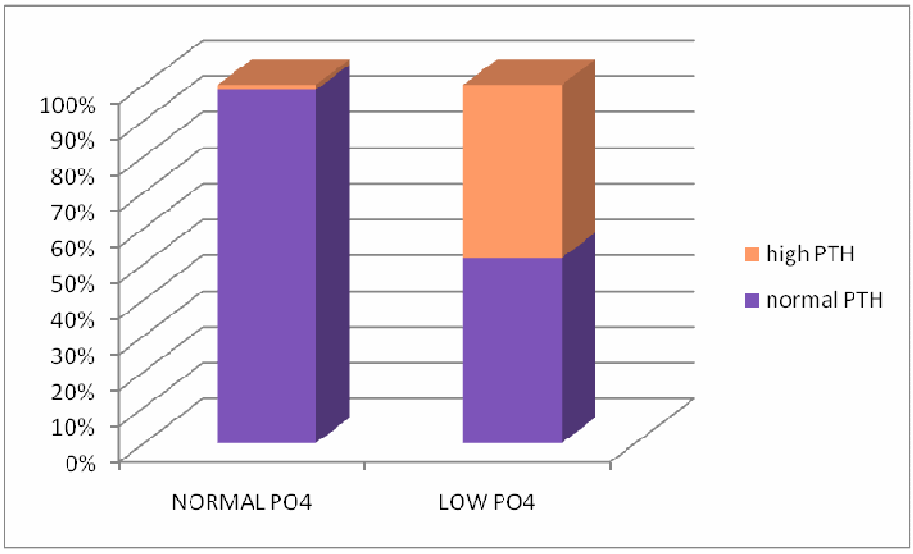


Hypophosphatemia was significantly associated with low serum levels of vitamin D.

TABLE - 19

Correlation of serum phosphorus and PTH

PTH Level		NormalPO_4	Low PO_4	Total	P value
Normal	Number	68	16	84	
	Percentage	81.0%	19.0%	84.0%	
High	Number	1	15	16	<0.001
	Percentage	6.3%	93.8%	16.0%	
Total		69	31	100	



Most patients with hypophosphatemia had elevated parathormone levels

DISCUSSION

DISCUSSION

The aim of the thesis was to study the prevalence of hypocalcemia among patients on chronic anti convulsant therapy (>6 months).

100 epilepsy patients attending the GGH, Chennai and fulfilling the criteria as mentioned in methodology were the subjects of this study. The prevalence of hypocalcemia among our study population was 32%. In a study done previously by Schmitt BP et al on 56 patients taking chronic anti convulsants, 29% (16 patients) were found to have hypocalcemia.[68]

The correlation between the number of anti convulsant drugs the patient taking and the presence of hypocalcemia was statistically analyzed. Among the patients on single drug therapy, the prevalence was 28.1%. Patients taking two drugs had a prevalence of 53.1% and for those on three drugs the value was 75%. Hence the number of anti convulsant drug usage correlated directly with the chance for hypocalcemia and the differences were statistically very significant ($p < 0.001$).

The duration of anti convulsant usage and whether that had a significant effect on the prevalence of hypocalcemia was analyzed. Those

taking drugs less than 12 months had a prevalence of 11.5%, while, of the patients taking drugs for more than 12 months, 81.3% had hypocalcemia. The difference when analysed statistically, was significant, with a p value < 0.001.

The serum levels of vitamin D was measured in all these patients and we found that, out of the 32 patients with hypocalcemia, 30 (91.3%) also had low levels of vitamin D. But in the normocalcemic group hypovitaminemia D was present only in 14.3% with the difference in prevalence being statistically significant (P< 0.001). In a similar study done on 56 patients taking chronic anti convulsants, Theodore J. Hahn et al reported that the serum calcium concentration of the patients correlated with serum vitamin D levels.[69]

Next the presence of low vitamin D levels in hypocalcemic patients was studied, and it was found that among the 23 patients of hypo vitaminosis D, 21 (91.3%) patients also had hypocalcemia and the finding was statistically significant.

The relationship between control of seizures and the presence of hypocalcemia was studied. It was found that, in patients with hypocalcemia, seizures control was poor, than in those with normocalcemia, with a P value of the difference being < 0.001. A similar finding was reported in the study done by Ali FE et al.[51] But of course,

sub therapeutic levels of anti-convulsants must have been ruled out, which, not being done, was one of the limitations of our study.

In this study, in addition to calcium levels, we also studied the correlation of serum Alkaline phosphatase levels with anti convulsant drug usage. Elevated Alkaline phosphatase levels was found in 25 of the 100 patients studied. Of these patients 19 also had hypocalcemia (76%) while the remaining 6 (24%) had isolated elevation of Alkaline phosphatase. In the study done by Schmitt BP et al, quoted previously, a similar finding was reported, with elevated ALP levels in 27% of the study group.[68]

The elevated Alkaline phosphatase levels were analyzed with respect to the duration of drug usage. With single drug use, the prevalence was 15.4% while with two and three drugs usage, the prevalences were 37% and 62.5% respectively. Thus more the number of drugs used, more the chances of elevated Alkaline phosphatase levels. Also the effect of duration of therapy on alkaline phosphatase levels was considered. Patients on drugs for less than 12 months had a prevalence of 15.4% while those taking drugs for more than 12 months had a greater prevalence of 35.4%. All the above mentioned findings were significant on statistical analysis ($p < 0.001$).

The coincidence of occurence of hypocalcemia and elevated ALP were analyzed. Of the 32 patients with hypocalcemia, 19(59.3%) also had elevated ALP. Isolated elevation of ALP alone was found in 6 (8.8%) patients.

The prevalence of elevated parathyroid hormone in patients with hypocalcemia was analyzed and was found to be present in half such patients, with statistical significance.

Also the correlation between low vitamin D and PTH levels was analysed and it was found that, of the 23 patients with low vitamin D,14 had elevated PTH levels, the number being significant on analysis with p value being <0.01.

Serum phosphate levels were done for the patients and it was found that hypophosphatemia was associated with either more number of, or longer duration of anti convulsant treatment and also with hypovitaminosis D and elevated parathormone levels. The associations were found to be statistically significant with $p<0.001$.

LIMITATIONS

1. The study was done on patients taking traditional anti convulsants. Hence the effect of newer drugs are not studied.

2. Only the total serum calcium was measured and not the ionised calcium which is the metabolically active component.

3. The serum levels of anti convulsants were not measured in patients, so as to attribute hypocalcemia, as the sole cause of poor seizure control.

4. The disease pathology causing seizures and its effect on seizure control were not analyzed.

5. While measuring alkaline phosphatase levels, only the total and not the bone iso-enzyme levels were measured.

6. Calcium and vitamin D supplementation and their effect on seizure control was not studied.

CONCLUSION

CONCLUSION

1. There is a significant prevalence of hypocalcemia in patients on chronic anticonvulsant therapy.

2. The number of anti convulsants used, as well as the duration of usage have a significant correlation with the prevalence of hypocalcemia.

3. Hypocalcemia in patients has a significant association with poor seizure control even with good drug compliance, when compared with normocalcemic patients.

4. Elevated Alkaline phosphatase levels were found in patients on chronic anti convulsant therapy either in isolation or in combination with hypocalcemia.

5. Elevated Alkaline phosphatase levels correlated significantly with the duration and number of anti convulsant drug usage.

6. Hence this study concludes that there is significant hypocalcemia and hypovitaminemia D in patients on chronic anti convulsant therapy and we suggest that calcium and vitamin D supplementation may be beneficial.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Sridharan R Moorthy BN, Prevalence And Pattern Of Epilepsy In India.Epilepsia 1999 May;40(5):631-636.

2. Theodore J.Hahn et al, AntiConvulsant DrugInduced Osteomalacia: Alteration In Mineral Metabolism And Responce To Vitamin D Administration , Calcified Tissue International 1979; 27:174-177.

3. Kruece K et al, The pathogenesis of anticonvulsant drug induced alteration of calcium metabolism: Eureopean Journal Of Paediatrics 1980; 133: 202-205.

4. Holick mf et al , photosynthesis of previtamin D3 in human skin and physiologic consequences, Science 1980 ; 210:203-205.

5. Thomas M, Demay M. Vitamin D deficiency and disorders of vitamin D metabolism. Endocrinology North Am 2000; 29:611-627.

6. Bikle DD, Gee E, Halloran B, et al. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin

and the vitamin Dbinding protein. J Clin Endocrinology Metab 1986; 63:954-959.

7. Bikle DD, Siiteri PK, Ryzen E, et al. Serum protein binding of 1,25-dihydroxyvitamin D: a Reevaluation by direct measurement of free metabolite levels. J Clinal Endocrinology Metab 1985; 61:969-975.

8. Nykjaer A, Dragun D, Walther D, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3 . Cell 1999; 96:507-515.

9. Takeyama K, Kitanaka S, Sato T, et al. 25-Hydroxyvitamin D3 1-hydroxylase and vitamin D synthesis. Science 1997; 277:1827-1830.

10. St-Arnaud R, Messerlian S, Moir J, et al. The 25-hydroxyvitamin D $_1$-hydroxylase gene maps to the pseudovitamin D deficiency rickets (PDDR) disease locus. J Bone Miner Res 1997; 12:1552-1559.

11. Fu G, Lin D, Zang M, et al. Cloning of human 25-hydroxyvitamin D_1 -hydroxylase and mutations causing vitamin D dependent rickets type 1. Mol Endocrinol 1997; 11:1961-1970.

12. Tanaka Y, DeLuca HF. The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. Arch Biochem Biophys 1973; 154:566-574.

13. Glass AR, Eil C. Ketoconazole-induced reduction in serum 1,25-dihydroxyvitamin D and total serum calcium in hypercalcemic patients, J Clin Endocrinol Metab 1988; 66:934-938.

14. Tanaka Y, Halloran B, Schnoes HK, et al. In vitro production of 1,25-dihydroxyvitamin D3 by rat placental tissue. Proc Natl Acad Sci USA 1979; 76:5033-5035.

15. Breslau NA, McGuire JL, Zerwekh JE, et al. Hypercalcemia associated with increased serum calcitriol levels in three patients with lymphoma. Ann Intern Med 1984; 100:174-178.

16. Barbour GL, Coburn JW, Slatopolsky E, et al. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 1981; 305:440-443.

17. Overbergh L, Decallonne B, Valckx D, et al. Identification and immune regulation of 25-hydroxyvitamin D-1 -alpha-hydroxylase in murine macrophages. Clin Exp Immunol 2000; 120:139-146.

18. Adams JS, Sharma OP, Diz MM, et al. Ketoconazole decreases the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia. J Clin Endocrinol Metab 1990; 70:1090-1095.

19. Adams JS, Diz MM, Sharma OP. Effective reduction in the serum 1,25-dihydroxyvitamin D and calcium concentration in sarcoidosis-associated hypercalcemia with short-course chloroquine therapy. Ann Intern Med 1989; 111:437-438.

20. Tanaka Y and Deluca HF. The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. Arch Biochem Biophys , 1973;154: 566–574.

21. Wu S, Finch J, Zhong M, Slatopolsky E, Grieff M, and Brown AJ. Expression of the renal 25-hydroxyvitamin D-24-hydroxylase gene: regulation by dietary phosphate. Am J Physiol Renal Fluid Electrolyte Physiol1996 271: F203–F208.

22. Chen KS and DeLuca HF. Cloning of the human 1 α,25-dihydroxyvitamin D-3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements. Biochim Biophys Acta 1995;1263: 1–9.

23. Ohyama Y, Ozono K, Uchida M, Shinki T, Kato S, Suda T, Yamamoto O, Noshiro M, and Kato Y. Identification of a vitamin D-responsive element in the 5'-flanking region of the rat 25-hydroxyvitamin $D_3$24-hydroxylase gene. J Biol Chem1994; 269: 10545–10550.

24. Ishizuka S and Norman AW. Metabolic pathways from 1α,25-dihydroxyvitamin D_3 to 1α,25-dihydroxyvitamin D_3-26,23-lactone. Stereo-retained and stereo-selective lactonization. J Biol Chem, 1987; 262: 7165–7170.

25. Ishizuka S, Kurihara N, Hakeda S, Maeda N, Ikeda K, Kumegawa M, and Norman AW. 1α,25-Dihydroxyvitamin D_3[1α,25-$(OH)_2D_3$]-26,23-lactone inhibits 1,25-$(OH)_2D_3$-mediated fusion of mouse bone marrow mononuclear cells. Endocrinology , 1988;123: 781–786.

26. Bischof MG, Siu-Caldera ML, Weiskopf A, Vouros P, Cross HS, Peterlik M, and Reddy GS. Differentiation-related pathways of 1α,25-dihydroxycholecalciferol metabolism in human colon adenocarcinoma-derived Caco-2 cells: production of 1α,25-dihydroxy-3epi-cholecalciferol. Exp Cell Res, 1998; 241: 194–201.

27. Brown AJ, Ritter C, Slatopolsky E, Muralidharan KR, Okamura WH, and Reddy GS. 1α,25-Dihydroxy-3-epi-vitamin D_3, a natural metabolite of 1α,25-dihydroxyvitamin D_3, is a potent suppressor of parathyroid hormone secretion. J Cell Biochem, 1999; 73: 106–113.

28. Nakagawa K, Sowa Y, Kurobe M, Ozono K, Siu-Caldera ML, Reddy GS, Uskokovic MR, and Okano T. Differential activities of 1α,25-dihydroxy-16-ene-vitamin D_3 analogs and their 3-epimers on human promyelocytic leukemia (HL-60) cell differentiation and apoptosis. Steroids , 2001;66: 327–337.

29. Baker AR, McDonnell DP, Hughes M, et al. Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci USA 1988; 85:3294-3298.

30. Germain P, Staels B, Dacquet C, Spedding M, Laudet V. "Overview of nomenclature of nuclear receptors". Pharmacol. 2006; 58 (4): 685–704.

31. Pettifor JM, Bikle DD, Cavaleros M, et al. Serum levels of free 1,25-dihydroxyvitamin D in vitamin D toxicity. Ann Intern Med 1995; 122:511-513.

32. Van Os CH. Transcellular calcium transport in intestinal and renal epithelial cells. Biochim Biophys Acta 1987; 906:195-222.

33. Wasserman RH, Fullmer CS. Calcium transport proteins, calcium absorption, and vitamin D. Annu Rev Physiol 1983; 45:375-390.

34. Karbach U. Paracellular calcium transport across the small intestine. J Nutr 1992; 122:672-677.

35. Fullmer CS. Intestinal calcium absorption: calcium entry. J Nutr 1992; 122:644-650.

36. Peng J, Chen X, Berger UV, et al. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J Biol Chem 1999; 274:22739-2746.

37. Kaune R, Munson S, Bikle DD. Regulation of calmodulin binding to the ATP extractable 110 kDa protein (myosin I) from chicken duodenal brush border by 1,25(OH)2 D3 . Biochim Biophys Acta 1994; 1190:329-336.

38. Wasserman RH, Chandler JS, Meyer SA, et al. Intestinal calcium transport and calcium extrusion processes at the basolateral membrane. J Nutr 1992; 122:662-671.

39. Harrison JR, Petersen DN, Lichtler AC, et al. 1,25-Dihydroxyvitamin D3 inhibits transcription of type I collagen genes in the rat osteosarcoma line ROS Endocrinology 1989; 125:327-333.

40. Price PA. Vitamin K dependent bone proteins. In Cohn DV, Martin TJ, Meunier PJ Calcium Regulation and Bone Metabolism: Basic and Clinical Aspects. Amsterdam, Exerpta Medica, 1987, pp 419-426.

41. Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/ osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95:3597-3602.

42. Kumar R et al, Vitamin D and calcium transport. Kidney Int 1991; 40:1177-1189.

43. Massheimer V, Fernandez LM, Boland R, et al. Regulation of Ca2+ uptake in skeletal muscle by 1,25-dihydroxyvitamin D3 ; role of phosphorylation and calmodulin. Mol Cell Endocrinology 1992; 84:15-22.

44. Pointon JJ, Francis MJO, Smith R. Effect of vitamin D deficiency on sarcoplasmic reticulum function and troponin C concentration of rabbit skeletal muscle. Clinical Science 1979; 57:257-263.

45. Harrison's principles of internal medicine 17th edition page2373-2377.

46. Text book of biochemistry,u.sathyanarayana , u.chakrapani 3 rd edition,page 123.

47. The washington manual of medical therapeutics 32 nd edition

48. Hoffman E: The Chvostek sign: a clinical study. Am J Surgery 1958;96:33–37.

49. Bushinsky DA, Monk RD: Calcium. Lancet 1998;352: 306–311.

50. Becker KL, ed: Principles and Practice of Endocrinology and Metabolism, 2nd ed. Philadelphia: J.B. Lippincott Company, 1995;618:1766–1767.

51. Ali FE, Al-Bustan MA, Al-Busairi WA, Al-Mulla FA,Loss of seizure control due to anticonvulsant-induced hypocalcemia. Ann Pharmacotherapy. 2004 Jun;38(6):1002-5.

52. Fitzpatrick LA, Pathophysiology of bone loss in patients receiving anticonvulsant therapy Epilepsy Behavioral. 2004 Feb;5 Suppl 2:S3-15.

53. Moro-Alvarez[a] Bone Disease Induced by Phenytoin Therapy: Clinical and Experimental study,M Eur Neurol 2009;62:219-230.

54. Hoikka V, Savolainen K, Alhava EM, Sivenius J, Karjalainen P, Parvianinen M,Anticonvulsant osteomalacia in epileptic outpatients. Ann Clin Res. 1982 Jun;14(3):129-32.

55. Lorraine A. Fitzpatrick Pathophysiology of bone loss in patients receiving anticonvulsant therapy epilepsy and behaviour feb 2004; 5:3-15.

56. Hahn TJ (1980) Drug-induced disorders of vitamin D and mineral metabolism. Clin Endocrinol Metab; 9 : 107-129,

57. Hahn TJ, Hendin BA, Scharp CR, Haddad JG Jr (1972b) Effect of chronic anticonvulsant therapy on serum 25-hydroxycalciferol levels in adults , N Engl J Med ; 287 : 900-904.

58. Jubiz W, Haussler MR, McCain TA, Tohnan KG (1977) Plasma 1,25- dihydroxyvitamin D levels in patients receiving anticonvulsant drugs. J Clin Endocrinol Metab; 44 : 617-621.

59. Melsen F, Norman AW (1979) Fractional intestinal calcium absorption in epileptics on anticonvulsant therapy. Acta Med Scand ;205 : 405-409.

60. Mosekilde L, Christensen MS, Lund B, S6rensen OH, Melsen F (1977) The interrelationships between serum 25-hydroxychole- calciferol, serum parathyroid hormone and bone changes in anti- convulsant osteomalacia. Acta Endocrinol (Kph); 84 : 559-565.

61. Melsen F, Norman AW (1979) Fractional intestinal calcium absorption in epileptics on anticonvulsant therapy. Acta MedScand ;` 205 : 405-409 .

62. "Reaction mechanism of alkaline phosphatase based on crystal structures. Two-metal ion catalysis". J. Mol. Biol. 218 (2): 449–64.

63. William's text book of endocrinology 10 th edition.

64. Kinetic analysis of parathyroid hormone clearance as measured by three rapid immunoassays during parathyroidectomy,clinical chemistry 48(1):1731-8.

65. Prahalad AK, Hickey RJ, Huang J, *et al*"Serum proteome profiles identifies parathyroid hormone physiologic response" (June 2006). Proteomics 6 (12): 3482–93.67)Poole.

66. K, Reeve J, "Parathyroid hormone - a bone anabolic and catabolic agent"2005 Curr Opin Pharmacol; 5 (6): 612–7.

67. The Parathyroid Glands and Vitamin D , Walter F., PhD. Boron (2003). Medical Physiology: A Cellular And Molecular Approaoch. Elsevier/Saunders. pp. 1300.

68. J Fam Pract. 1984 Jun;18(6):873-7. Prevalence of hypocalcemia and elevated serum alkaline phosphatase in patients receiving chronic anticonvulsant therapy -Schmitt BP, Nordlund DJ, Rodgers LA.

69. Theodore J. Hahn, M.D., Barry A. Hendin, M.D., Cheryl R. Scharp, B.A., Vincenza C. Boisseau, B.A., and John G. Haddad, Jr., M.D. Serum 25-Hydroxycalciferol Levels and Bone Mass in

Children on Chronic Anticonvulsant Therapy,N Engl J Med 1975; 292:550-554.

ANNEXURE

PROFORMO

Calcium homeostasis abnormalities in chronic anticonvulsant therapy

Name :
Age :
Sex :
Hospital number :
Drugs used and duration : 1.
2.
3.

Indication for anticonvulsive therapy :
Other risk factors for bone disease :
Compliance & Seizure control :
History of bone fractures :
Clinical examination :
Height: weight: BMI:

Investigations	
Complete Hemogram	
Liver function tests	
Renal function tests	
Serum Vit D2	
Urine Routine	
Serum Calcium	
Serum Phosphorus	
Serum Alkaline phosphatase	

PATIENT CONSENT FORM

Title
Calcium homeostasis abnormalities in chronic antiepileptic therapy.

Study centre : Govt.General hospital, MMC, Chennai
Patient's name :
Patient's age :
Identification number :

I confirm that I have understood the purpose of the procedures of the above study. I have had the opportunity to ask questions and all my questions have been answered satisfactorily. ☐

I understand that my participation in the study is entirely voluntary and that I am free to withdraw from the study at any time without my legal rights being affected ☐

I understand that sponsors of the study, others working on sponsor's behalf, the ethics committee and the regulatory authorities will not need my permission to look at my health records both in respect of current study and any future research that may be conducted in relation to it; even if I withdraw from the study I agree to this access. However I understand that my identity will not be revealed in any information released to third parties unless required by law. I agree not to restrict the use of any data or results arising from the study. ☐

I agree to take part in the above the study an to comply with the instructions given during the study and inform about any change in my health status to the investigator. ☐

I hereby give permission to undergo complete clinical examination and investigations as part of the study. ☐

Signature of the patient

Patient's name and address place date

Signature of the investigator

Investigator's name place date

MASTER CHART

S.No.	AGE	SEX	DIAGNOSIS	DRUGS	DURATION	CALCIUM-C	PHOSPHORUS	ALP	VIT D	PTH	ALBUMIN	SEIZURE CONTROL
1.	15	F	M	P	8 M	10.2	2.8	48	40.4	60	4.2	Y
2.	40	F	PSS	P	14 M	7.8	2.3	262	9.4	108.3	3.8	N
3.	18	F	N	P	12 M	10.8	3.2	46	56.6	28.6	4	Y
4.	59	M	MR	P	9 M	9.4	4	68	37.2	32.5	4.2	Y
5.	19	M	TBM	P	14 M	8.2	2.2	288	30.6	20.4	3.8	Y
6.	32	M	CPS	C	12 M	9	4	48	14.8	44.6	3.6	Y
7.	37	F	M	P+PB+S	12 M	7.6	2.4	202	8.8	382.4	3.8	Y
8.	27	F	M	P+S	20 M	7.8	2.2	252	8.6	180.4	4	Y
9.	40	F	PSS	P+S	11 M	9.4	3.1	62	18.4	42.4	3.8	Y
10.	38	M	PTS	P	12 M	10.6	3.6	42	26.4	45.4	3.6	Y
11.	25	M	MR	P	10 M	9.8	3.4	46	34	20.6	3.8	Y
12.	12	F	TBM	P+S	7 M	7.8	2.1	212	9.6	121.6	4.2	N
13.	24	M	A	S	9 M	10.4	4	54	24	18.6	3.6	Y
14.	20	M	I	P	8 M	8.8	4.2	38	16.8	24.8	3.6	Y
15.	17	F	CPS	C	24 M	8	2.4	158	9.6	134.6	4	Y
16.	19	M	M	P	15 M	9	3.2	246	32.5	40.6	4	Y
17.	32	F	I	S	20 M	8.2	2.2	78	19.6	43	3.6	Y
18.	18	F	N	S	11 M	9.8	3.8	108	32	28.2	3.8	Y
19.	29	M	I	P+PB	9 M	7.8	2	192	8.6	232.4	3.6	Y
20.	19	F	I	P	13 M	7.6	2.4	282	8.4	168.4	4.4	Y
21.	17	F	I	S	10 M	10.6	3.6	122	20	38.5	4	Y
22.	20	M	CPS	C	7 M	10.6	3.4	68	18.4	54	4.2	Y
23.	39	M	PSS	P+S	22 M	8.2	2	88	20.2	108.6	3.8	Y
24.	28	M	I	P	10 M	10.4	3.6	48	10.8	38.4	4	Y
25.	17	M	N	P+C+S	16 M	8.4	2.1	128	10.8	44.2	4.2	Y
26.	22	F	I	S	11M	10.2	4	56	35.6	28.6	4.2	Y
27.	16	F	CPS	C+S+P	6 M	9.6	4.2	78	30	42.4	4	Y
28.	40	M	TBM	P+S	12 M	7.8	2.2	156	9.6	142.4	4.4	N
29.	33	M	I	S	10 M	9.4	3.6	40	26.6	20.6	3.8	Y
30.	31	F	N	C	12 M	10.2	3.2	196	30.6	42.5	4	Y

S.No.	AGE	SEX	DIAGNOSIS	DRUGS	DURATION	CALCIUM-C	PHOSPHORUS	ALP	VIT D	PTH	ALBUMIN	SEIZURE CONTROL
31.	17	M	MR	P	7 M	8.4	2.4	224	28.6	46	4	N
32.	16	M	MR	P	9 M	9.8	3.6	84	20.4	34.6	4.2	Y
33.	20	M	I	P+PB+S	16M	7.6	2.4	320	9.8	126.2	4	Y
34.	31	M	M	C+S	17 M	8.2	2	68	40.4	28.6	4.4	Y
35.	37	M	N	P	12 M	9.2	4.1	108	38.2	34.6	3.8	Y
36.	28	M	CPS	C	10 M	9.8	3.6	44	28	42	4	Y
37.	30	F	I	C	18 M	10.4	4.2	48	44	36.5	3.8	Y
38.	27	M	I	P+S	16 M	8	3.4	188	8.4	88	4.2	Y
39.	36	M	I	P	16 M	10.2	2.6	56	36.6	38.6	4	Y
40.	41	M	I	P	24 M	7.8	2.3	286	24	42.6	4	Y
41.	30	M	T	S	7 M	10.8	4	48	42	34.6	4	N
42.	22	M	CPS	C	19 M	10.4	3.4	96	24.6	36	4.1	Y
43.	39	F	M	P+S	14 M	8.2	2.3	56	9.4	46	4	Y
44.	16	F	CPS	C	8 M	9.6	3.6	240	20.6	42.4	4.2	Y
45.	17	M	I	P	10 M	9	3.2	64	30.8	32.6	4	Y
46.	22	M	TBM	S+C	11 M	8	2.4	56	31.8	36	3.6	Y
47.	29	F	I	P	12 M	9.4	2.8	88	28.6	34.5	3.8	Y
48.	16	F	I	S	14 M	9.6	3.2	80	30.8	34.6	4.4	Y
49.	28	M	M	P+B	13 M	8.2	2.4	64	9.6	64	4	Y
50.	20	M	A	P	17 M	10.2	2.8	134	40.6	42	4.2	Y
51.	38	F	M	P+B	14 M	10.4	3.2	256	37.5	28.4	4	Y
52.	22	M	A	P+S	17 M	8	2	204	8.8	242.2	4.2	Y
53.	46	M	PSS	P+S	18 M	7.8	2.4	288	9.6	134.2	4	N
54.	22	F	A	S	7 M	10.4	4.2	60	23.6	40.4	3.8	Y
55.	39	F	I	P	9 M	10.4	4.2	78	31.5	24	3.6	Y
56.	31	F	I	P	16 M	8.4	2.3	84	34.4	26	4.4	N
57.	39	F	I	S	11 M	9.6	3.2	44	26.6	38.6	4.2	N
58.	15	F	PSS	S	9 M	9.2	3.8	46	34.6	24	4.2	Y
59.	18	F	I	P+PB+S	18 M	8.2	2.4	96	8.4	86.4	4	N
60.	35	F	N	P+S	23 M	8.2	2	196	9.4	66.4	3.8	Y
61.	38	F	I	P+S	11 M	9.6	4	202	37.8	38.6	3.8	N
62.	45	M	I	P	6 M	10.4	4.2	86	23.8	40.4	3.6	N

S.No.	AGE	SEX	DIAGNOSIS	DRUGS	DURATION	CALCIUM-C	PHOSPHORUS	ALP	VIT D	PTH	ALBUMIN	SEIZURE CONTROL
63.	27	M	I	P	8 M	10.2	4.2	80	44	36.4	3.6	Y
64.	29	M	M	P	19 M	8	2.1	188	32.4	38	4.4	N
65.	18	F	MR	S	10 M	9.6	3.6	62	40	45.4	4.2	Y
66.	32	F	M	P+PB	9 M	9.2	3.4	42	33.6	32.6	4.2	N
67.	17	F	N	P+PB	13 M	8	2	208	9.2	56.4	4	N
68.	29	M	A	P	8 M	10.4	3.8	88	43.5	48.4	4	Y
69.	25	M	I	C	7 M	10.8	3.6	76	26.4	36	3.6	Y
70.	14	F	I	S	18 M	9.6	4.4	54	29.4	42	3.8	Y
71.	43	M	TBM	S	18 M	10.4	4.2	64	38.6	46.4	3.8	Y
72.	33	M	M	P+S	23 M	8.2	2.4	98	9.4	304.2	4.4	Y
73.	20	F	A	S	11 M	8.8	4	112	28.4	42.4	4.2	Y
74.	31	M	TBM	P+S+PB	18 M	10.2	3.8	36	34.6	42	4	N
75.	38	M	M	P+S	24 M	8.2	2	124	9.6	46.8	4	N
76.	42	M	TBM	P	13 M	9.8	3.4	48	42.8	45.6	3.6	Y
77.	56	F	M	P	20 M	9.4	3.2	130	38	40.6	4.2	Y
78.	54	M	PSS	P	7 M	10.4	3.6	64	28.4	42.6	4.2	Y
79.	20	F	N	S	13 M	9.2	4	84	42	38	4.2	Y
80.	38	F	M	P+S	18 M	9.6	4.2	44	32.6	26.8	4	Y
81.	18	F	I	P+PB+S	24 M	7.8	2.4	246	9.8	238.2	3.8	N
82.	38	M	I	P	10 M	10.2	4.2	46	38.6	42	3.6	Y
83.	32	M	I	S	14 M	10.8	3.8	56	18.4	42.6	3.6	Y
84.	30	F	I	P+S	10 M	9.4	3.4	66	16.8	45.6	4	Y
85.	24	F	A	P+S	13 M	10.8	3.2	56	33.6	44.2	4	Y
86.	24	F	A	S	19 M	8.8	3.7	42	28.6	48	4.2	N
87.	35	F	M	P+PB+S	17 M	8.1	2	224	32.4	33.6	4.2	N
88.	15	F	MR	S	11 M	9.4	2.8	68	26.4	40.2	4	Y
89.	19	F	PTS	P	10 M	9.8	3.8	52	44	38.4	3.6	Y
90.	37	F	EDH	P+S+PB	8 M	10.4	3.4	212	28	38	3.8	Y
91.	32	M	N	P	13 M	9.6	4.2	80	32.6	48.4	4.2	Y
92.	22	F	T	P+S	20 M	10.6	4.3	46	40.2	56.4	4	Y
93.	39	F	I	P	15 M	8.8	4.2	74	37.4	46.4	4.4	Y
94.	45	M	M	P+S	6 M	10.4	3.6	80	16	42.2	3.8	Y

S.No.	AGE	SEX	DIAGNOSIS	DRUGS	DURATION	CALCIUM-C	PHOSPHORUS	ALP	VIT D	PTH	ALBUMIN	SEIZURE CONTROL
95.	38	M	TBM	P	11 M	9.2	3.4	46	28.6	40.4	3.6	Y
96.	27	F	I	S	12 M	9.6	3.4	246	34.6	44	3.6	Y
97.	29	F	I	P	8 M	10.2	4.2	44	44	28.4	4.2	Y
98.	26	M	I	P+S	22 M	8.2	2.3	124	54	24	3.8	N
99.	31	M	M	P	10 M	10.8	3.4	56	28.6	42.4	4.4	Y
100.	42	F	I	P	11 M	10.4	3.4	60	24.6	48.2	4.2	Y

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-600 003

Telephone 25363970
Fax 044 2535115
Dated : 12.05.2010

L.Dis.No.14597/ME5/Ethics Dean/MMC/2010

Title of the work : "Calcium Homeostasis Abnormalities in Patients on Chronic Anticonvulsant Therapy."

Principal Investigator : Dr. N. Govindarajan.
Designation : PG in MD General Medicine.
Department :
Madras Medical College & GGH, Ch-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 12th May 2010 at 2.p.m in Pharmacology Seminar Hall, Madras Medical College, Chennai -3

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
4. You should not deviate form the area of the work for which you applied for ethical clearance.
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulation of the institution(s).
7. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, MMC,CHENNAI

CHAIRMAN
IEC,MMC, CHENNAI

DEAN
MADRAS MEDICAL COLLEGE,
CHENNAI -3